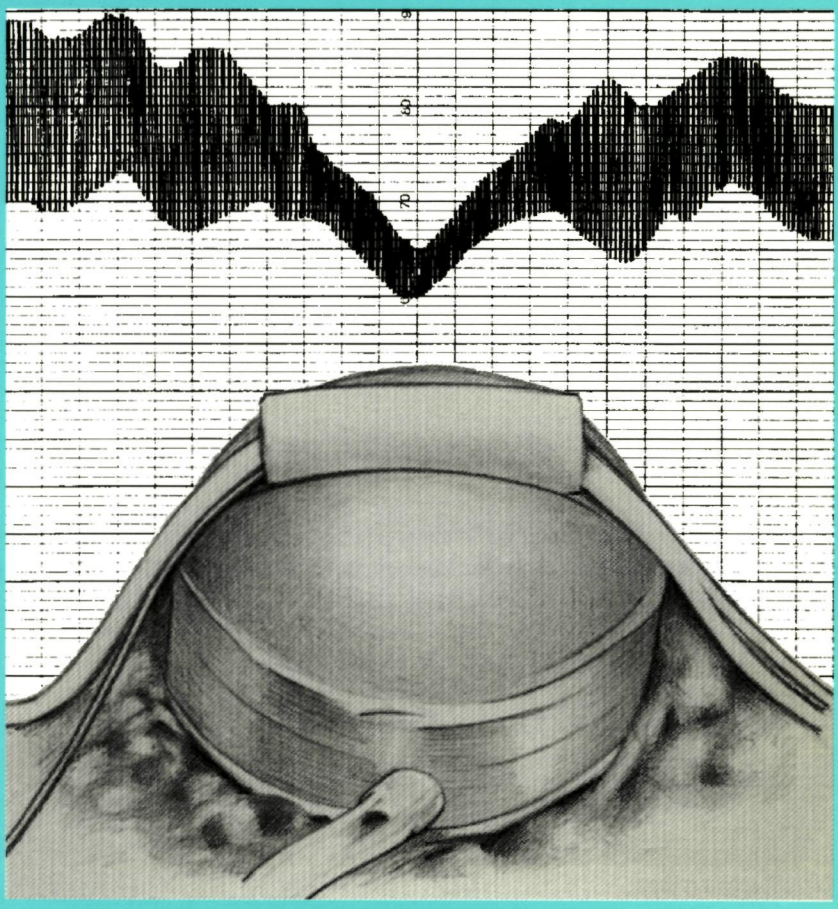
Peripheral nerve elongation by laser Doppler flowmetry controlled expansion



L.P. van der Wey

PERIPHERAL NERVE ELONGATION

BY LASER DOPPLER FLOWMETRY CONTROLLED

EXPANSION

AN EXPERIMENTAL STUDY IN RABBITS

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AN EXPERIMENTAL STUDY IN RABBITS

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van de Medische Wetenschappen

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To my parents

To Suzanne

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CONTENTS

CHAPTER 1

INTRODUCTION

Functional recovery after nerve repair is influenced by a complex pattern of many cellular and biochemical events initiated at the time of injury. The surgeon operating upon and caring for patients with peripheral nerve injuries must have a clear understanding of both the anatomy of the nerve and the neurobiology of nerve degeneration and regeneration. The surgeon's primary goal is to help achieve correct reinnervation of peripheral targets as rapidly as possible. His role is restricted to provide optimal mechanical alignment of the corresponding fascicles. The functional results can be influenced positively by timing the operation optimally, careful handling of the damaged nerve ends and choosing the best method of nerve repair. The past three decades have shown great improvements in instrument manufacture and the availability of superior magnifying devices. Introduction of the operating microscope and refinements in microneurosurgical techniques have evidently improved the functional results of peripheral nerve repair. Moreover, interfascicular nerve grafting enabled the surgeon to reconstruct peripheral nerve defects without tension at the suture line. However, despite these advances, the functional results of nerve grafting are still far from excellent, and harvesting of the donor nerve graft is associated with donor-site morbidity of scarring, sensory loss, and occasionally, a painful neuroma.

Tissue expansion is a relatively new technique and has been applied for reconstruction of large skin and soft tissue defects. If a peripheral nerve is elongated by the use of a tissue expander, the additional length can be used to overcome a nerve defect and enable a delayed primary repair with a single coaptation. Moreover, donor-site morbidity associated with nerve grafting is avoided. Nerve expansion and repair may yield better functional results since only one coaptation is involved. However, it is important to control nerve function during expansion. Nerve function and structure depend intimately on the integrity of its blood supply. The present study was designed to investigate whether peripheral nerve elongation by means of a tissue expander is possible with preservation of nerve function and structure when nerve blood flow is controlled during expansion. The assessment and treatment of nerve injuries is based on a clear understanding of the anatomy and physiology of peripheral nerve, the fundamental characteristics of which are presented in chapter 2. In chapter 3 current techniques of peripheral nerve repair are reviewed. The specific aims

of the present study are summarized in chapter 4. In chapter 5 laser Doppler flowmetry (LDF) is evaluated as a method of monitoring nerve blood flow during expansion. The functional and neurophysiological aspects of peripheral nerves elongated by LDF controlled expansion are studied in chapter 6. Chapter 7 reports on the morphological changes in peripheral nerves subjected to LDF controlled expansion. The relationship between functional, neurophysiological and morphological parameters of peripheral nerves elongated by LDF controlled expansion is elucidated in chapter 8. In chapter 9 the results of the present study are summarized and evaluated. In addition, suggestions for further studies on nerve expansion and repair are presented.

CHAPTER 2

ANATOMY AND PHYSIOLOGY OF THE PERIPHERAL NERVE

2.1 General

Peripheral nerves consist of bundles of nerve fibers with their associated supporting tissues and vascular supply (Fig. 1 and 2). The nerve fibers are extended processes of cell bodies situated in the spinal cord (motor neurons), dorsal root ganglia (sensory neurons) or sympathetic ganglia (sympathetic neurons). Each fiber is embedded in loose connective tissue, the *endoneurium*. Many nerve fibers are collected to form a fascicle. These fascicles are surrounded by a multilamellar sheath, the *perineurium*. The perineurium has selective permeability and is essential for maintaining an optimal endoneurial environment. Fascicles are embedded in the *epineurium*. The epineurium consists of well-vascularized areolar connective tissue and protects nerve fibers from compression and stretch deformation. Whereas earlier studies attribute the elastic properties of the nerve to the endoneurium (Schneider 1952) or the perineurium (Sunderland and Bradley 1961), later studies indicate that the elasticity of the nerve trunk depends mainly upon the epineurium, much less on the perineurium and only a little on the endoneurium (Haftek 1970). The vascular supply to the nerve is provided by a longitudinally oriented intraneural microvascular system with abundant anastomoses located throughout all connective tissue layers (Lundborg and Dahlin 1991, Thomas et al. 1993).

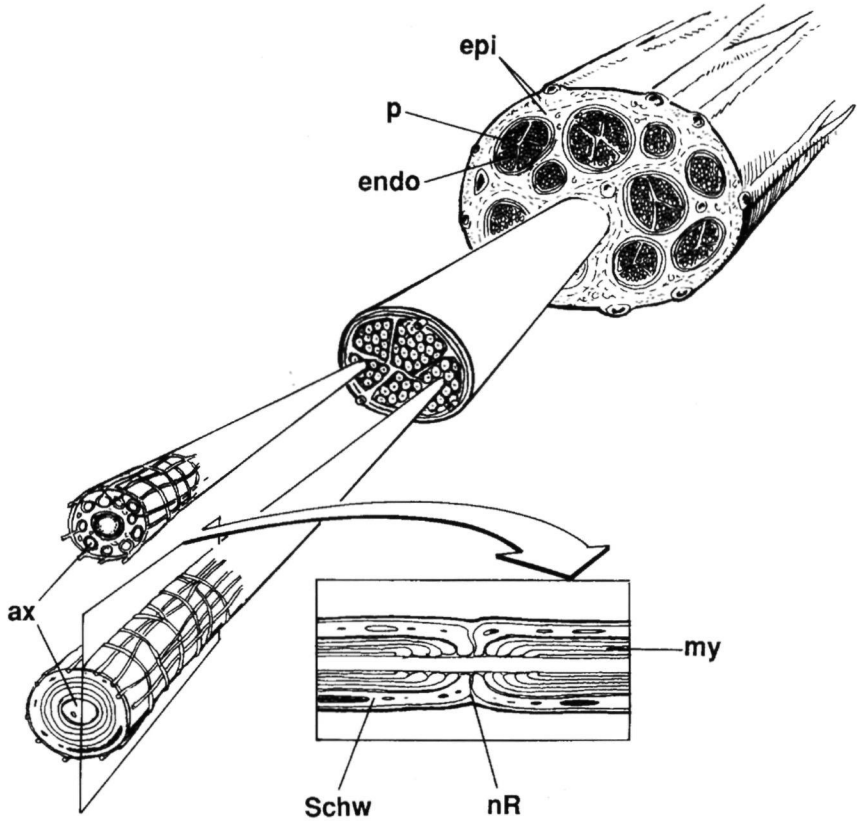

Fig. 1. Microanatomy of the peripheral nerve (see text for details): epi: epineurium; p: perineurium; endo: endoneurium; ax: axon; Sch: Schwann cell; my: myelin sheath; nR: node of Ranvier.

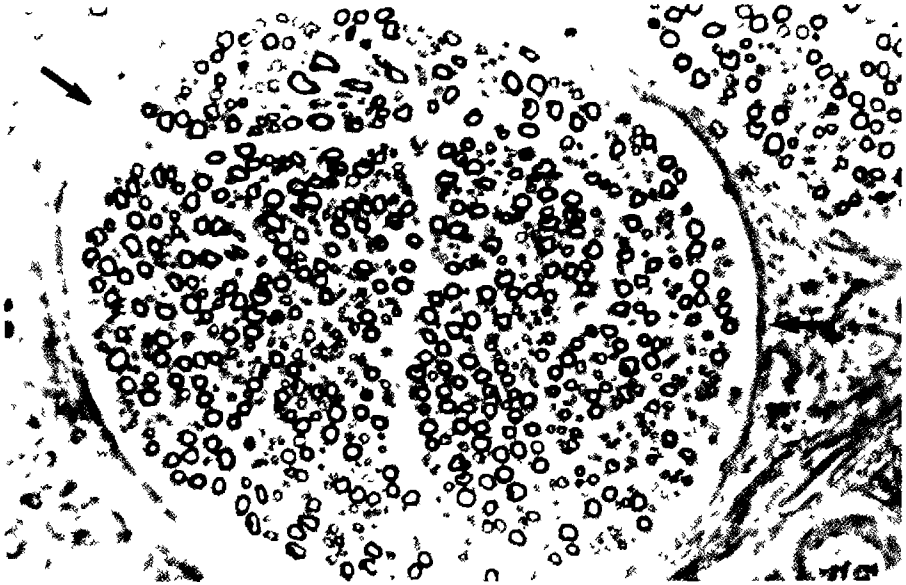

Fig. 2. Transverse section through fascicle of human peripheral nerve. A population of large and small myelinated fibers is ensheathed by the perineurium (↑). The endoneurium comprises the intrafascicular connective tissue. Areolar connective tissue of the epineurium surrounds the fascicle. x 195.

2.2 Nerve fibers

The nerve fiber or axon contains as much as 90% of the total axoplasmic volume of the neuron. The axon depends on the nerve cell body for both survival and function. The cytoskeleton, the most conspicuous of the axoplasmic components, consists of *microtubules, neurofilaments,* and *microtrabecular matrix* (Ellisman 1981, Ellisman and Porter 1980, Tsukita et al. 1982). It includes the machinery necessary for continuous bidirectional axoplasmatic transport (Hollenbeck 1989, Thomas et al. 1993). Along the axon anterograde transport of structural proteins and organelles necessary for its maintenance, growth and metabolic activity occurs. These axonal organelles include mitochodria, endoplasmic reticula, neurofilaments, microtubules and dense particles. There is also a retrograde transport of information via proteins

back to the cell body. Both anterograde and retrograde transport are energy-requiring functions (Bray 1985). Nerve fibers are either *myelinated* or *unmyelinated*. The myelinated nerve fiber consists of one axon and a set of Schwann cells that are arranged serially along the outside of the axon (Fig. 3 and 4).

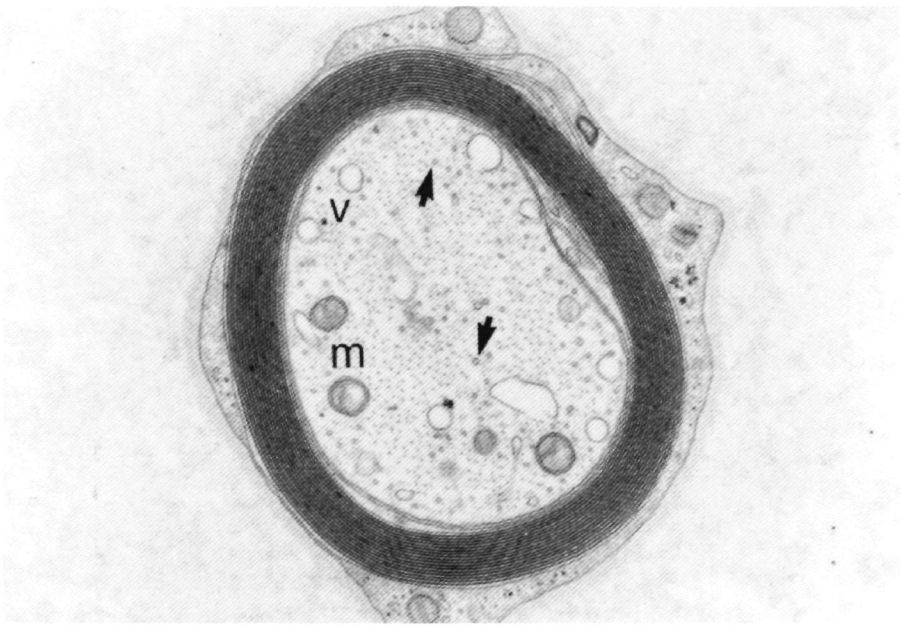

Fig. 3. Transverse section of myelinated axon. The axoplasm contains microtubuli (↑), neurofilaments, mitochondria (m) and vesicles of smooth endoplasmic reticulum (v). The myelin sheath is composed of condensed, concentrically wrapped Schwann cell membranes. The dark major dense lines arise by fusion of the inner surfaces of the Schwann cell membrane; the less dense intraperiod lines arise from the fusion of the outer surfaces. The Schwann cell cytoplasm is visible external to the myelin sheath. x 26,500.

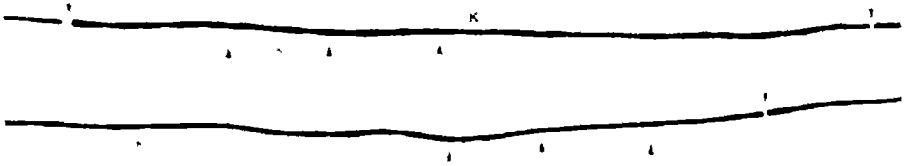

Fig. 4. Teased myelinated fibers. The nodes of Ranvier (↑) represent the exposed axon membrane in between the serially arranged Schwann cells, which have formed myelin segments or internodes. There is a linear relationship between fiber diameter and internodal length: thicker fibers have longer internodes. The Schwann cell nuclei are located in the middle of a segment and are visible as slight indentations of the myelin sheath (K). x150.

Each Schwann cell enwraps the axon segment with which it is associated with a multilayered cell membrane specialization, the *myelin sheath* (Lundborg and Dahlin 1991, Thomas et al. 1993). Schwann cells approach each other and interdigitate approximately every millimeter along the fiber, allowing a 1-μm region of excitable membrane to be exposed to the extracellular fluid. At these locations, known as the *nodes of Ranvier* (Fig. 5), there is an exchange of ions between the axon and the surrounding extracellular fluid, allowing for the rapid saltatory propagation of impulses in myelinated fibers (see 2.3).

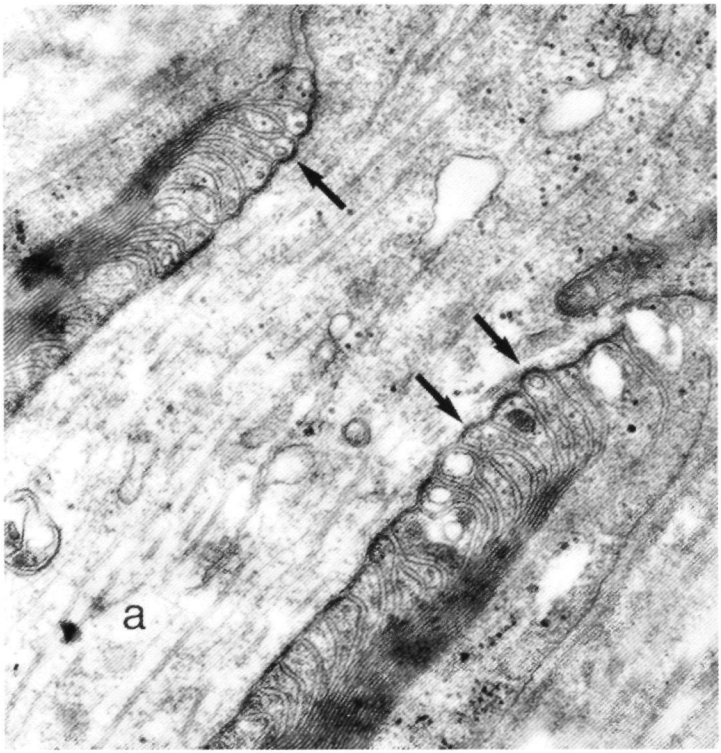

Fig. 5. Longitudinal section through node of Ranvier. In the paranodal region terminal loops of myelin lamellae, which contain little Schwann cell cytoplasm, are attached to the axolemma with specialized dense contacts (↑). The axon (a) contains microtubuli, neurofilaments, mitochondria and vescicular structures. x29,000.

In unmyelinated fibers, Schwann cells accumulate several numbers of axons, which are located in internal troughs. In these fibers the ion exchange is continuous, resulting in a lower conduction velocity (Galbraith and Myers 1991). The Schwann cell and its contents is bounded peripherally by a continuous plasma membrane and its basal lamina (Asbury and Johnson 1978). This basal lamina and the reticular and collagen fibrils of the endoneurium provide a framework of support for the axon, the *endoneurial tube* (Lundborg and Dahlin 1991).

2.3 Saltatory conduction

Myelination of axons with a regular interruption at the nodes of Ranvier is the elegant solution of nature to optimize nerve function in the sense of obtaining a maximal propagation velocity along the axon given its diameter (Fig. 6).

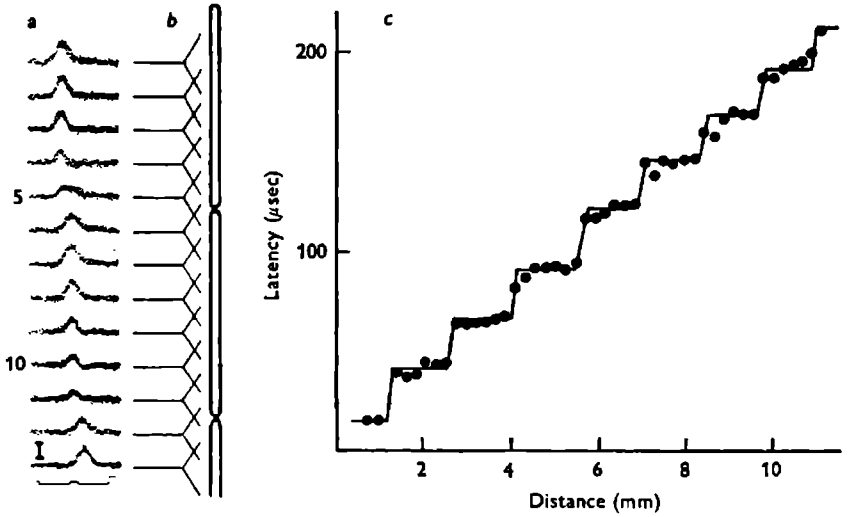

Fig. 6. Saltatory propagation along a myelinated nerve fiber.
a: successive records of external longitudinal current from a single fiber as electrodes were moved along ventral root.
b: lines from each record indicate positions of electrodes with respect to diagrammatic fiber.
c: latency to peak of external longitudinal current as a function of distance along the fiber.
(From Rasminsky and Sears (1972) Internodal conduction in undissected demyelinated nerve fibres. J Physiol 227:323-350, with permission).

The first convincing evidence for this "saltatory" conduction was given by Tasaki and colleagues (Tasaki et al. 1953). The characteristics of saltatory impulse conduction are dependent on specific properties of the myelinated axon and its (patho)physiological variations. These dependencies are studied quantitatively in both experimental demyelinating

neuropathies (Rasminsky and Sears 1972, Smith et al. 1982) and mathematical models (Koles and Rasminsky 1972, Waxman 1980). The latter studies support the proportional relationship between conduction velocity and axon diameter in normal fibers. Internodal distance and myelin thickness can be altered drastically after a process of demyelination and remyelination. Conduction velocity appears to be remarkably insensitive to changes in internodal distance, but decreases significantly as a result of a reduction in myelin thickness (Waxman 1980). Loss of myelin without disruption of the axon-myelin sheet primarily increases membrane capacitance and slows depolarization and impulse conduction. Even complete loss of myelin over several internodes does not necessarily block conduction. Propagation block is especially found in acute demyelinating processes (Bostock 1993). The transitions between (still) myelinated and demyelinated segments of a nerve fiber are the potential loci for such conduction block (Waxman and Brill 1978). The influence of paranodal demyelination on impulse conduction appears strongly dependent on the properties of the exposed paranodal axolemma. Disruption of the axon-myelin sheet attachment, which normally seals off the internodal from the nodal axolemma, reduces the effective nodal resistance and can block conduction without any significant myelin loss (Bostock 1993). Within wide limits, the influence of the paranodal length *per se* is not essential for conduction safety and conduction velocity (Waxman 1980).

2.4 Perineurium

The fascicles are surrounded by a dense, mechanically strong multilamellar sheath, the perineurium (Fig. 7) (Key and Retzius 1876).

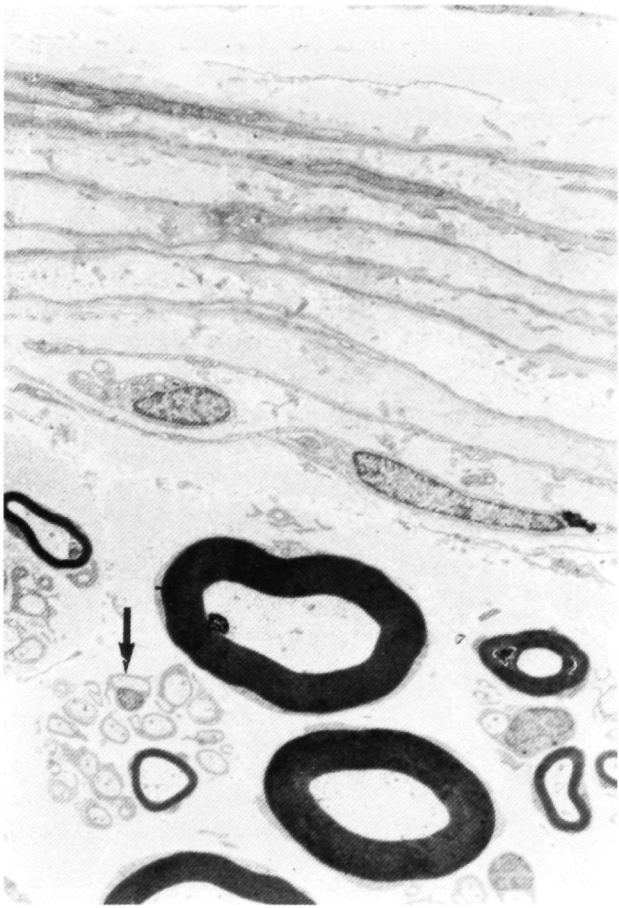

Fig. 7. Transverse section through perineurium and some subperineurial fibers. The multilamellar perineurium is composed of flattened perineurial cells, which are bounded on either side by basal lamina. Two large and some smaller axons are surrounded by myelin sheaths. One to several unmyelinated axons are invested by one Schwann cell (↑). x 1850.

The perineurium is composed of flattened cells bounded on both sides by basement membrane (Thomas 1963). The number of lamellae varies with the diameter of the fascicle. Up to 15 layers are present around the fascicles of mammalian nerve trunks (Thomas et al. 1993). At their margins, contiguous perineurial cells are linked by tight junctions (Beamish et al. 1991). They constitute the morphologic basis of the perineurial diffusion barrier (Thomas et al. 1993). The perineurial cell lamellae are separated by clefts. The clefts contain longitudinally and obliquely oriented collagen fibers and elastic fibers (Thomas and Jones 1967). The perineurium is traversed by blood vessels linking the longitudinal anastomotic network of arterioles and venules in the epineurium with the longitudinal intrafascicular capillary network (Lundborg 1970). The perineurium is normally under tension circumferentially because it contains an interstitial environment with a slightly positive fluid pressure (+1.2-1.5 mm Hg) (Myers et al. 1978). Positive *endoneurial fluid pressure* results in bulging of endoneurial contents at the free nerve ends ('*mushrooming*') after perineurial transection. The perineurium is a mechanically strong membrane protecting the endoneurial contents. In addition, the perineurium has selective permeability and is one component of the blood-nerve barrier, the other component being the endothelial tight junctions of capillaries in the endoneurium. The blood-nerve barrier corresponds to the bloodbrain barrier and is essential for maintaining a unique endoneurial environment (Lundborg and Dahlin 1991).

2.5 Epineurium

The epineurium is a loose connective tissue layer embedding and protecting the fascicles (Bloom and Fawcett 1975). It contains a well-developed vascular plexus with numerous longitudinally oriented blood vessels that feed the endoneurial capillary network. The outer layers of the epineurium are condensed to a sheath (epifascicular epineurium) investing the whole nerve trunk, whereas the inner layers (interfascicular epineurium) extend between the fascicles and keep them loosely together (Millesi and Terzis 1984). The relative amount of epineurium is increased when nerves cross joints and in nerves located superficially in an extremity, probably due to the extra mechanical requirements at these locations (Sunderland 1978). The epineurium

provides a certain amount of gliding of nerve with movement of the extremity. The excursion of nerves ranges from 6.8 mm for the ulnar and median nerve distal to the carpal tunnel, to 15 mm for the brachial plexus (Wilgis and Murphy 1986). The longitudinal movement of nerve trunks is made possible by an adventitia of loose areolar tissue between nerve and surrounding tissues. The interfascicular epineurium enables motion beween individual fascicles (Millesi 1986). The gliding function is inhibited by scarring associated with nerve injury (Lundborg 1988).

2.6 Vascularization

Both impulse conduction and axonal transport require an adequate energy supply, which is provided by intraneural microvessels. Peripheral nerves are well-vascularized structures with separate but extensively interconnected microvascular systems in the epineurium, perineurium and endoneurium (Fig. 8).

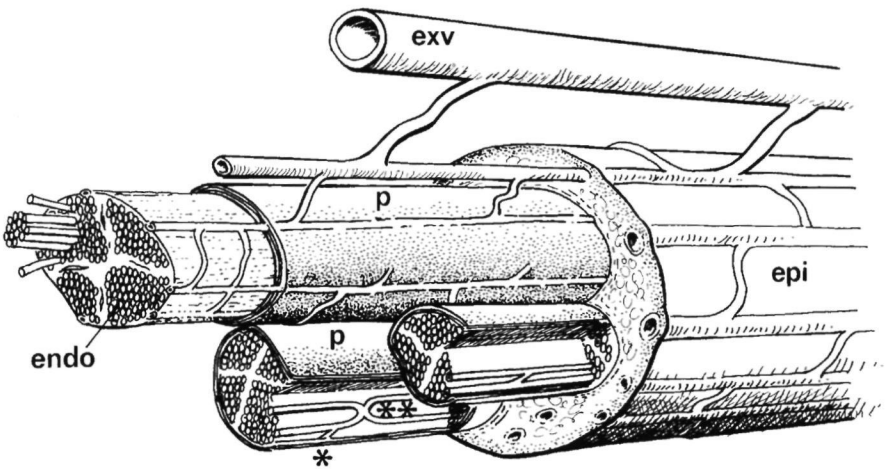

Fig. 8. Vascularization of the peripheral nerve (see text fot details): exv: extrinsic vessels; epi: epineurium; p: perineurium; endo: endoneurium. Note the oblique course of vessels penetrating the perineurium (*) and an endoneurial double loop formation (**).

There is an *intrinsic system* consisting of vascular networks in the epi-, peri- and endoneurium and an *extrinsic system* involving segmental regional vessels approaching the nerve trunk at various levels along its course (Sunderland 1945, Lundborg 1970, 1975, 1979, Bell and Weddel 1984). The intrinsic and extrinsic systems are in equilibrium with each other, each being able to compensate for functional disturbances in the other (Lundborg and Brånemark 1968, Lundborg 1970, 1975, 1979). The epineurial vessels anastomose intimately with the vascular plexus in the perineurium. Longitudinal microvessels course over long distances between the perineurial lamellae before piercing the inner perineurial layer in a characteristic oblique way (Lundborg 1970, 1975, 1979, Myers et al. 1986). Within the fascicles there is an endoneurial microvascular plexus consisting mainly of large capillaries but also including some arterioles and venules (Lundborg 1970, 1975, 1979, Bell and Weddell 1984). No single direction of blood flow in endoneurial vessels is predominant. Blood flow may change direction immediately as a result of slight compression to the nerve's surface or damage to a regional feeding artery (Lundborg and Brånemark 1968, Lundborg 1970, 1975). The intraneural longitudinal collaterals of the intrinsic system provide a considerable margin of safety to the nerve trunk when the extrinsic vessels are excluded by mobilization of the nerve (Lundborg 1970, 1975). Other factors adding to the resistance to ischemia include the high basal nerve blood flow relative to the metabolic needs of nerve and the ability of peripheral nerve to utilize anaerobic metabolism (Low 1984, McManis et al. 1993). Longitudinal tension to a nerve trunk interferes with intraneural microvascular flow at fairly low levels of strain (Lundborg and Rydevik 1973). If tension is applied to a nerve, regional segmental vessels are stretched and extrinsic vascular inflow is inhibited. Moreover, tension narrows fascicles, resulting in compression of intrafascicular capillaries. Impairment of venular flow is noted at 8 percent stretch of the rabbit tibial nerve. At 15 percent elongation the flow in all intraneural vessels ceases entirely (Lundborg and Rydevik 1973, Ogata and Naito 1986).

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CHAPTER 3

TECHNIQUES OF PERIPHERAL NERVE REPAIR

3.1 Historical review

Galen (130-200 A.D.) was the first to document the differences between nerves and tendons (Brock 1929). It was widely believed that nerves could regenerate spontaneously and that partial injury or manipulation of the severed ends would cause convulsions. As early as the seventh century, Paulus Aegineta used a combination of agglutination and sutures to attempt repair (MacKinnon and Dellon 1988a). Arabic physicians in the ninth and tenth centuries apparently attempted surgical repair of sectioned nerve ends with primitive sutures (Brock 1929). Surgical repair of a peripheral nerve has been reported as early as 1608 (Ferrara 1608). Save for these early attempts, little progress was made in the repair of nerve injuries until the nineteenth century. Early techniques of nerve repair now appear inappropriate: turning back a nerve flap created from the distal portion of the nerve in a proximal direction, suturing this to a similar flap turned down in a distal direction from the proximal end of the nerve (Letievant 1872, Mackenzie 1909). Stookey (1919) subsequently demonstrated no regeneration of nerve tissue using this nerve flap, and therefore the technique was abandoned. Other techniques included: cutting the nerve ends tangentially in order to increase the surface area for nerve contact (Markoe 1885), a side-to-side suture of the nerve ends (Rawa 1885) and a suture transfixion technique similar to the technique used to repair tendons (Snyder et al. 1968, Spitzy 1917, Hirschel 1915, Heinemann 1916). Letievant (1872) described a nerve implantation experiment by inserting the distal stump of the divided nerve between the intact nerve fascicles of the proximal portion. Hueter in 1873 described repair of nerves by sutures placed in the epineurial sheath. This technique, with some refinement, remained the standard of nerve repair until the recent past. Early results from nerve suturing were very poor, most likely from suture line infection, inadequate debridement, and excessive tension at the suture line. Davis and Cleveland (1934) have reviewed the history of nerve repair. Six different techniques of nerve repair have been described: nerve implants (Letievant 1872, Hoffmann 1884), nerve flaps (Letievant 1872), "suture à distence" (Assaky 1886), tubulation (Gluck 1880), nerve crossing (Flourens 1828) and nerve grafts (Davis and Cleveland 1934). Davis and Cleveland advocated the use of nerve grafts in the treatment of nerve gaps. Various types of tubular structures have been suggested to help bridge the gap that

could not be managed effectively with simple suture techniques. These have included rubber tubes (Garriety 1955), fascial sheaths (Kirk and Lewis 1915), freeze dried arteries (Hirisawa and Marmor 1967, Weiss 1943), collagen (Braun 1966, Madison et al. 1985), tantalum (Weiss 1944) and polyglactin (Molander et al. 1982, Seckel et al. 1986a). Gluck in 1880 was the first to describe a tubulation technique, using the central canal of decalcified bone as a pathway for nerve ends.

Early experimental studies in nerve grafting dated back to the nineteenth century. Philipeaux and Vulpian described the first experiment of nerve grafting in 1870 (Philipeaux and Vulpian 1870). In 1878 Albert described the first clinical use of a nerve graft (Albert 1878a). The first successful nerve graft was reported by Mayo-Robson (1917). Nerve grafts were rarely used until Seddon (1947) introduced the technique of cable grafting to fill large nerve gaps. Renewed interest in autografting was caused by the improved results with cable grafts (Seddon 1947, 1963). This technique prevented the central necrosis of large grafts by allowing more rapid revascularization of smaller caliber cable grafts. Results of peripheral nerve repair were significantly improved after the introduction of microsurgical techniques and Millesi's contribution of tension-free interfascicular nerve grafting (Millesi et al. 1972, 1976). Taylor and Ham described the first free vascularized nerve graft in 1976, thus supplying another technique in the management of peripheral nerve defects (Taylor and Ham 1976).

3.2 Operative nerve repair

The purpose of all nerve repair techniques is to restore continuity of the nerve trunk in order to achieve optimal reinnervation of end organs. According to Millesi and Terzis (1984), the four overriding principles of nerve repair are as follows:

1. Preparation of the nerve stumps, often with removal of portions of the epineurium and separation of individual or groups of fascicles, until all visible signs of damage have been debrided.
2. Approximation, with special reference to the nerve gap and the amount of tension at the suture line.
3. Coaptation of the nerve ends with attention to bringing the cross-section of the fascicles into optimal contact under appropriate rotational alignment. This may be by fascicle, groups of fascicles, or the entire stump.
4. Maintenance of reapproximation using mechanical means, i.e., suture, blood clotting, fibrin, or glue.

Proper fascicular identification is mandatory in performing a successful nerve repair. One of the most common reasons for unsuccessful nerve repair is the failure of the surgeon to resect back to normal fascicular structure in both the proximal and distal nerve stumps. Once this resection is accomplished, the surgeon must study the situation individually to determine which type of nerve repair is possible. No single repair method is appropriate for every instance of nerve transection. The nerves in the arm undergo changes in their course from the brachial plexus to the fingertips: polyfascicular in the upper arm, oligofascicular in the elbow region and monofascicular in the hand. The monofascicular and polyfascicular patterns lend themselves most readily to an epineurial repair, whereas an oligofascicular nerve is best repaired using an interfascicular technique (Millesi 1990).

3.2.1 Epineurial repair

Coaptation of the nerve stumps by suturing the external epineurium is a classic method of nerve repair (Fig. 1). An important step is the initial preparation of the nerve edges. The nerve must be transected perpendicular to its axis so that a uniform flush surface is produced. Fascicles then mushroom from the neural stumps, and the epineurium retracts slightly. The epineurial vessels and fascicular pattern of the cut ends

should be identified under high magnification to ensure correct matching of the ends when the suture is performed. Placement of a key suture at an external landmark provides rotational alignment, and the remaining stitches are placed more or less in a random fashion. The sutures are tied carefully so that the faces are flush but not compressing. The advantage of the epineurial suture technique is its simplicity and the minimal dissection trauma required. The main disadvantage is that it is a whole nerve suture. An epineurial suture that looks adequate from the outside, may hide from view severe mismatch and malalignment of the fascicles. Despite these possible drawbacks, a carefully performed epineurial repair often produces a satisfactory functional outcome (Lundborg 1988, Mackinnon and Dellon 1988a, Millesi 1990, Gelberman 1991).

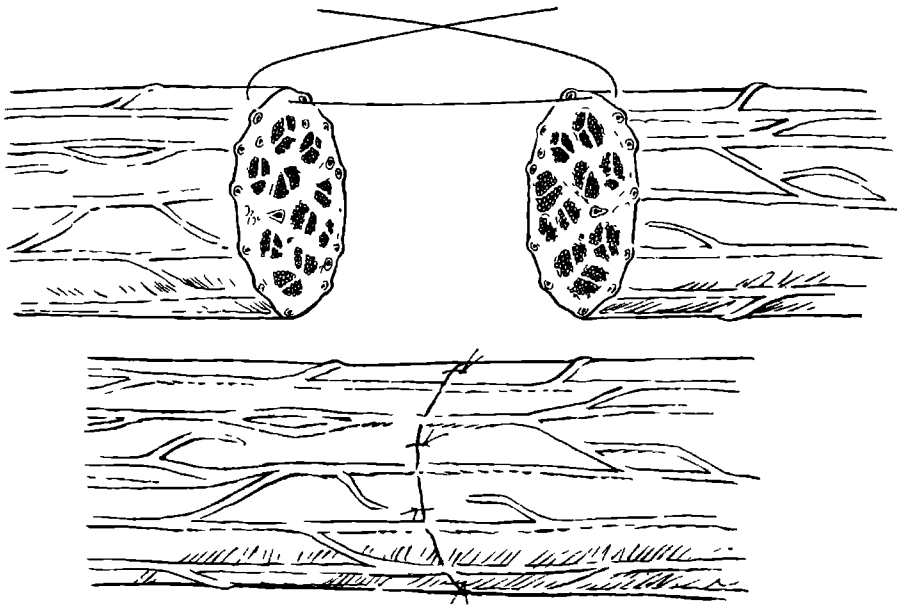

Fig. 1. Epineurial repair (see text for details).

3.2.2 Fascicular repair

Perineurial repair was first advocated by Langley and Hashimoto (1917), and later by Sunderland (1953). Fascicles or groups of fascicles are carefully freed by microsurgical dissection, and are matched to their counterparts (Fig. 2). Coaptation is maintained using perineurial sutures. The advantage of fascicular repair is the possibility of achieving an optimal realignment of corresponding fascicular components and regrowth of axons into the appropriate endoneurial channels. However, fascicular repair requires increased surgical manipulation and consequently possible internal disruption and scar formation. Moreover, sutures penetrating the perineurium may induce microherniation of endoneurial contents and may delay restoration of an optimal environment for axonal growth (Lundborg 1988, Mackinnon and Dellon 1988a, Millesi 1990, Gelberman 1991).

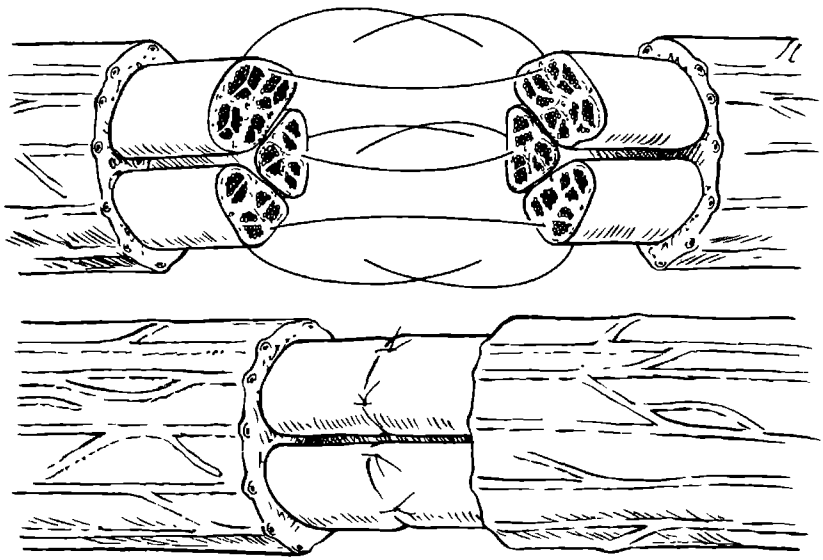

Fig. 2. Fascicular repair (see text for details).

3.2.3 Epineurial versus fascicular repair

There is no consistent evidence suggesting that one repair technique is superior over the other. Analysis of the retrograde transport of horseradish peroxidase in repaired rat sciatic nerve reveals improved specificity of muscle reinnervation after fascicular repair when compared to epineurial repair (Brushart 1980a, 1980b, 1981). While one study reported a slight advantage of perineurial suture over suture of the epineurium (Orgel and Terzis 1977), in other studies no distinct advantage of perineurial suture over epineurial repair could be found (Cabaud et al. 1976, 1980, Kline et al. 1981). The potential benefits of fascicular alignment are probably not experienced clinically because of the increased surgical manipulation required in order to execute a fascicular repair and the potential for mismatching of the fascicles. Although the epineurial repair is not as exact, it may allow for neurotropic and neurotrophic factors to exert their influence on the direction of nerve fiber growth. However, the question of epineurial versus fascicular nerve repair is an incorrect question. There are situations in which only epineurial nerve repair can be performed and other situations in which fascicular nerve repair certainly has its merits. The most important factors influencing the choice of methods are the fascicular architecture of the nerve, the level of injury, the relative amounts of fascicular and epineurial tissue, and the timing of surgery. The level of injury is important because generally, at the most proximal levels, fibers of different peripheral branches of a nerve are widely scattered throughout the fascicle, with each fascicle containing fibers from most or all of the distal branches. This type of plexus formation is less evident more distally, as fibers of distal branches become more concentrated in separate fascicles occupying particular sections of the nerve. Therefore, the suture of separate fascicles is generally reserved for distal levels of the arm. The goal of the nerve repair will be to align appropriate fascicles with minimal surgical trauma to the nerve. In general, an acute cleanly severed nerve that demonstrates near complete fascicular correspondence is best treated with an epineurial repair. If a particular fascicle is identified in both nerve stumps as having a specific function, a fascicular repair for that fascicle is indicated. Thus, treatment of the motor component and the sensory portion of the ulnar nerve as separate nerves has been demonstrated superior. Often a combination of the two suture techniques is used. Epineurial sutures can be placed to assure

strength and maintain orientation of the structures, while perineurial stitches can be applied to secure definite fascicular groups in position (Lundborg 1988, Mackinnon and Dellon 1988a, Millesi 1990, Gelberman 1991).

3.3 Sutureless methods of nerve repair

3.3.1 Glue

Young and Medawar (1940) first described the technique of glueing as a method of sciatic nerve repair in rabbits and dogs. Subsequently this method was applied for the approximation of the median nerve in a patient. Tension at the repair site was a contraindication to the use of this method (Seddon and Medawar 1942). Fibrin glue performed well when used to maintain apposition of nerve grafts without tension. If the repair is subjected to any tension, a few epineurial stay sutures are added or a nerve graft is used. Fibrin glue is applied as a thin layer four times longer than the diameter of the nerve in order to create a cylinder sufficient to resist shear. Foil wrapped around the nerve repair site for 30 seconds ensures even distribution of the fibrin glue and separation from surrounding tissues. Accidental glue interposition inhibited neural regeneration histologically (Hamm et al. 1988). Bertelli and Mira (1993) described a technique of sciatic nerve repair in rats of freezing to trim and fibrin glue to coat the nerve. The observed axonal alignment was superior to that obtained by microsuture alone. A comparison of primary fibrin glue repair and nerve grafting to microsuture in patients resulted in a significant improvement in results based on subjective criteria and a significant reduction in operative time (Egloff and Narakas 1983, Narakas 1988).

3.3.2 Laser

Argon or low-output carbon dioxide (CO_2) laser beams are by itself not able to bond transected nerve stumps. They are effective by converting nonadherent into adherent material: respectively by conversion of blood erythrocytes into an adhesive coagulant after absorption by porphyrine derivatives (argon) or by creation of a fibrin film adhesive after absorption in the surface of the fibrin film (CO_2). CO_2 laser-assisted nerve repair involves insertion of two stay sutures for approximation of the nerve ends, wrapping of several layers of fibrin

around the suture site and irradiation of the entire surface of the film with the laser beam. The fibrin film is then adhesive enough to withstand some tension at the suture site and the stay sutures are removed. The advantages of laser repair include improved regeneration of nerve fibers, diminished axonal sprouting because the suture site is sealed, and reduced scarring around the suture site on histologic examination (Almquist 1988). Argon laser has been used in the repair of peripheral nerves in the animal model. When compared to conventional microsurgical techniques, improved results were seen both histologically and functionally (Campion et al. 1990). Nerve repairs by CO_2 laser coagulation were less resistent to traction than those by means of epineurial sutures (Thumfart et al. 1990). This is in concurrence with more recent work demonstrating that CO_2 laser repair was not effective in secondary nerve reconstructions unless tension at the repair site was reduced (Dubuisson and Kline 1993).

3.3.3 Cell surgery

Painstakingly performed sutures may look satisfactory on the outside, yet histologic examination of conventional nerve repairs shows a catastrophic internal picture after both epineurial and perineurial sutures. In contrast, loose approximation of stumps by fibrin clot or tubulation has little disruptive effect on the structure. Statistical analysis of results of nerve repair suggests that neurite regrowth after nerve repair is random, and not influenced by current methods of repair (de Medinaceli and Rawlings 1987). Imperfect repairs and dubious coaptation often have satisfactory results, while sophisticated microsurgical repairs are not always rewarded with good functional recovery. On the other hand, exact coaptation is worth seeking: a crush lesion has perfect stump adaptation and optimal functional recovery. While other types of surgery involve suturing tissues, peripheral nerve surgery essentially is surgery of a cell process, the axon. These aspects have led to a new concept of peripheral nerve surgery: 'cell surgery'. The precision of repair at the cellular level is enhanced by minimising physical and chemical damage to the stumps and by ensuring stress-free stump abutment. Trimming of nerve stumps is necessary because the tips are always damaged by the initial trauma. Nerves are difficult to cut because of the contrast between the strength of the envelopes and the fragility of contents. Hardening the stumps through briefly

freezing results in a smooth surface of transection with minimal crush injury. However, trimming pokes new holes in the cell membrane resulting in a new chemical injury. The adverse affects of ion shifts are avoided by soaking the nerve stumps in a modified Collins fluid. The addition of chlorpromazine protects the nerve against the adverse affects of free calcium ions, and the high potassium content helps minimise the stress of freezing. Coaptation of the stumps without stress is accomplished by using an anti-retraction device according to the principle of de Saint-Venant (de Saint-Venant 1856). Additional protection against unwanted displacements is provided by the minuscule fibrin clot that develops spontaneously around the stumps and consolidates the repair. A preliminary report of the application of this technique in humans indicated encouraging short-term results (de Medinaceli and Merle 1991, Merle and de Medinaceli 1992).

3.4 Management of nerve defects

3.4.1 General

A nerve defect reflects to the amount of actual nerve tissue that is lost and a nerve gap refers simply to the distance between the two nerve ends. The extent of a nerve gap is influenced by the defect of nerve tissue, the retraction of the nerve stumps, the actual joint position and the resection of damaged tissue from the two nerve stumps (Millesi 1986). Direct suture of the ends of a severed or lacerated nerve is not always possible without significant tension. Even slight tension interferes with intraneural blood flow, compromising the nutrition of cellular components at both nerve ends. Moreover, tension reduces the transectional area of the fascicle, thereby increasing normal endoneurial fluid pressure, which is detrimental to axonal flow of nutrients (Lundborg 1988). In addition, the regenerating axons can be damaged even after they have already crossed the suture line by hypertrophy of scar tissue and by stretching of the scar (Millesi 1973). Thus, complete absence of tension at the suture site is regarded as the most important factor for a successful nerve repair. When an end-to-end nerve repair cannot be carried out with the assurance that there is no tension at the suture site, a nerve graft is needed to bridge the gap. Nerve regeneration after grafting without tension is much better than after direct end-to-end suture under moderate tension (Millesi et al. 1972, Terzis et al. 1975). The role of the nerve graft is to provide a mechanical framework

as well as an optimal neurochemical environment for the advancing axonal sprouts (Mackinnon and Dellon 1988a). Currently, peripheral nerve defects are best treated with interfascicular nerve grafts, performed with the aid of microneurosurgical techniques (Millesi et al. 1972, 1976). The transectional surfaces are studied under high magnification and corresponding fascicular groups are united by individual nerve grafts (Fig. 3).

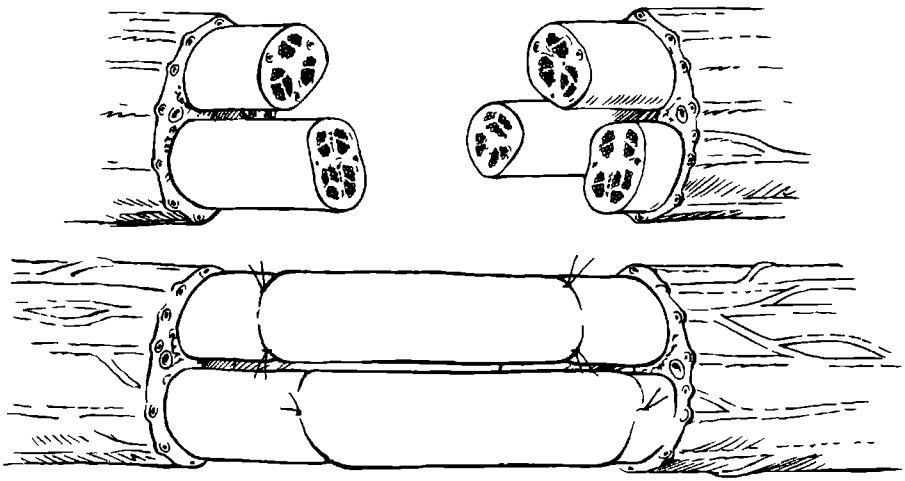

Fig. 3. Interfascicular nerve grafting (see text for details).

3.4.2 Autologous nerve graft

The autologous nerve graft is the workhorse for the management of peripheral nerve defects. Selection of a donor nerve graft takes into consideration the cross-sectional area of the recipient nerve, the total length of nerve defect and donor site morbidity. The most commonly used donor nerve is the sural nerve (30 to 40 cm). Other sources of donor nerve are the lateral femoral cutaneous nerve, and in the upper extremity the medial antebrachial cutaneous nerve, the lateral antebrachial cutaneous nerve, or the dorsal cutaneous branch of the ulnar nerve. An autologous nerve graft behaves like a distal stump after it is removed from the donor nerve. The Schwann cells of the grafts and

their basal lamina play an essential role. Axon cells will die out in the process of Wallerian degeneration, but the Schwann cell-lined tubules will survive to accomodate proximal axonal sprouts if the graft is adequately revascularized. Revascularization of the nerve graft occurs through the formation of adhesions at the recipient site, resulting in fixation of the nerve graft. Consequently, the nerve graft cannot adapt to different positions of the limb by passive movement. If an autograft fails due to ischemia, the Schwann cells do not survive and fibrosis develops (Lundborg 1988). The use of pedicled or free tissue transfer will enhance revascularisation of autologous nerve grafts in scarred recipient beds (Merle and Dautel 1991).

3.4.3 Vascularized nerve graft

Strange (1947) reported the possibility of grafting the median nerve by staged transposition of the pedicled ulnar nerve. Taylor and Ham (1976) introduced the concept of free vascularized nerve grafts. The vascularized nerve graft has the advantage that it has less tendency to sclerose in comparison to conventional nerve grafts. Conventional nerve grafts are always submitted to ischemia for a few days. Moreover, the vascularized nerve graft does not develop adhesions except at the site of coaptation. Consequently, vascularized nerve grafts are less sensitive to longitudinal traction (Millesi 1986). Some experimental studies showed an improved rate of axonal regeneration in vascularized nerve grafts compared to nonvascularized nerve grafts (Koshima and Harii 1985, Restrepo et al. 1985), whereas in others no difference could be demonstrated (McCullough et al. 1984, Seckel et al. 1986b). However, in those experiments examining function, results were superior in vascularized nerve grafts (Shibita et al. 1988, Kanaya et al. 1992). Use of vascularized nerve grafts is indicated in severely scarred recipient beds (Breidenbach and Terzis 1984, Breidenbach 1988). However, as the vascularity of the bed improves, the indications for a vascularized nerve graft become more controversial (Breidenbach and Graham 1991). Vascularized nerve grafts may also be indicated for reconstruction of proximal nerve lesions. Clinical studies report encouraging results of vascularized nerve grafts in brachial plexus reconstruction (Bonney et al. 1984). The vascularized ulnar nerve graft has been used in the reconstruction of supraclavicular brachial plexus injuries with results superior to those of conventional sural nerve graf-

ting (Birch et al. 1988). Doi and coworkers (1987, 1992) successfully used the vascularized sural nerve graft for peripheral nerve reconstruction. A vascularized deep peroneal nerve graft may be the procedure of choice in secondary digital nerve reconstruction across scarred beds or following injuries with poor soft-tissue viability (Rose and Kowalski 1985, Rose et al. 1989, Koshima et al. 1991).

3.4.4 Nerve allograft

Albert performed the first human nerve allograft in 1878 without reported follow-up (Albert 1878b). The effectiviness of peripheral nerve regeneration across nerve allografts depends on the histocompatibility status of donor and host. Attempts to decrease the rejection of nerve allografts and hence improve axonal regeneration has focussed on either nerve-graft pretreatment or recipient immunosuppression. Pretreatment with high-dose irradiation or lyophilization decreased the host recognition of donor allografts (Mackinnon et al. 1984a). However, regeneration across these pretreated grafts was poor (Mackinnon et al. 1984b). In contrast, immunosuppression of the host animal with cyclosporin A resulted in regeneration across nerve allografts comparable to that occurring across a nerve autograft (Bain et el. 1988, 1992, Fish et al. 1992). Immunosuppressive therapy was necessary only for the period of time required for host axons to traverse the nerve allograft (Midha et al. 1993). Permanent survival is possible if the Schwann cells and the fibroblasts of the allograft are gradually replaced by cells of the recipient nerve (Millesi 1986, Ishida et al. 1993). In view of possible side-effects of immunosuppressive treatment, nerve allografts should only be used in the selected patient with an otherwise irreparable nerve injury (Mackinnon and Hudson 1992).

3.4.5 Muscle interposition

Peripheral nerve fibers have the ability to grow into skeletal muscle when it degenerates (Sanes et al. 1978). Muscle grafts are easily obtained and simply prepared. The basement membrane matrix of skeletal muscle has a tubular configuration resembling that of peripheral nerves (Glasby et al. 1986, 1990). Moreover, the basement membrane tubes are large enough to accomodate even the largest nerve fibers (Norris et al. 1988). Freezing in liquid nitrogen and thawing in distilled water renders the muscle cells dead and disrupted without structural

damage to the basement membrane matrix (Glasby et al. 1986). Muscle graft represents a technically more simple method of repairing a large mixed nerve than inserting a cable nerve graft (Glasby et al. 1990). Experimental studies suggest that the muscle graft may be a useful technique in nerve repair (Glasby 1991). Treated muscle autografts were successful in peripheral nerve repair in rats and non-human primates with functional reinnervation of the target muscles (Gattuso et al. 1988, 1989). Autogenous muscle grafts provided good sensory and motor recovery in the treatment of granulomatous lesions in peripheral nerves of guinea pigs with experimental leprosy (Pereira et al. 1990). Good regeneration through denatured skeletal muscle grafts bridging gaps in the ulnar nerve has been observed in primates (Glasby et al. 1986). In patients, digital nerve repairs with muscle grafts gave superior results when compared with conventional nerve sutures (Norris et al. 1988, Pereira et al. 1991). However, reconstruction of mixed motor/sensory nerves using freeze-thawed muscle autografts yielded generally poor results (Calder and Norris 1993).

3.4.6 Tubulation

Tubulation is a method that requires insertion of the proximal and distal nerve stumps into a hollow tube. Sutureless tubulation techniques have been reported since Gluck in 1880 used the central canal of decalcified bone as a pathway for nerve ends. Various types of tubular structures have been suggested: fascial sheaths (Kirk and Lewis 1915, Platt 1919), rubber tubes (Garriety 1955), freeze dried arteries (Weiss 1943, Hirisawa and Marmor 1967), collagen (Braun 1966, Madison et al. 1985), tantalum (Weiss 1944) and polyglactin (Molander et al. 1982, Seckel et al. 1986a). Evidence of rodent peripheral nerve regeneration has been shown through silicone tubes (Gibson and Daniloff 1989), collagen tubes (Colin and Donoff 1984), expanded polytetrafluoroethylene tubes (Cuadros and Granatir 1987), mesothelial tubes (Lundborg and Hansson 1980), pseudosynovial tubes (Lundborg and Hansson 1980) and biodegradable polyester tubes (Seckel et al. 1984). The critical length of the nerve gap in the rodent model was 10 mm or less (Seckel et al. 1984). A laminin-containing gel (Madison et al. 1985) or collagen (Rosen et al. 1990) as an extracellular matrix stimulates peripheral nerve regeneration in vivo. While addition of a collagen extracellular matrix resulted in an axonal regeneration of the artificial nerve

graft equal to sutured autografts as measured by axonal counts, physiological and functional methods at 11 or 12 months (Rosen et al. 1990), this was not evident at 17 to 21 months (Rosen et al. 1989). Vascularized pseudosheath tubes as well as bioabsorbable polyglycolic acid (PGA) tubes were used in bridging nerve gaps up to 3 cm with nerve regeneration comparable to that across sural nerve grafts in primates (Mackinnon and Dellon 1988b, Dellon and Mackinnon 1988). Human peripheral digital nerve was able to regenerate across gaps of up to 3 cm (mean 1.7 cm) in length when facilitated by a PGA conduit (Mackinnon and Dellon 1990).

3.4.7 Vein interposition

Büngner was the first to report repair of a lacerated nerve with a blood vessel (Büngner 1891). Foramitti found that formalin-fixed artery was much more resistant to absorpton than fresh artery in bridging a nerve gap in the sciatic nerve (Foramitti 1904). Nerve does indeed regenerate through the lumen of a vein. Autogenous vein graft can serve as a conduit for a regenerating nerve (Chiu et al. 1982). However, vein grafts were inferior to nerve grafts in bridging nerve defects measuring more than 10 mm in rabbits (Rigoni et al. 1983). Several modifications of this technique have been described (Brunelli et al. 1993, Tang et al. 1993, Wang et al. 1993). Inside-out vein grafts showed improved axonal regeneration compared to standard vein graft conduits (Wang et al. 1993). Vein grafts filled with muscle yielded funtional results similar to those of conventional nerve grafts (Brunelli et al. 1993). In patients, autogenous vein grafts were effective when applied to bridge small gaps of 3 cm or less in peripheral sensory nerves (Walton et al. 1989, Chiu and Strauch 1990). When normal nerve slices were inserted inside the vein graft, good functional results were obtained after reconstruction of substantial digital and ulnar nerve defects (Tang 1993, Tang et al. 1993).

3.4.8 Direct muscular neurotization

Direct muscular neurotization may provide another adjunct in the treatment of peripheral nerve injuries (Brunelli and Monini 1985). Muscle reinnervation by direct implantation of a nerve into the muscle tissue results in the formation of new motor end-plates (Brunelli 1991). This

technique has been used both experimentally and clinically with encouraging results (Brunelli 1991, Brunelli and Brunelli 1993, Mackinnon et al. 1993). Direct muscular neurotization offers an alternative to tendon transfer or arthrodesis when the distal motor nerve is destroyed and conventional nerve grafting is not possible (Wyrick and Stern 1992, Brunelli and Brunelli 1993, Mackinnon et al. 1993).

3.4.9 Nerve elongation

A peripheral nerve can be elongated preoperatively by the use of a tissue expander (Manders et al. 1987). Tissue expansion is a relatively new technique and has been used to provide local tissue for reconstruction of skin and soft tissue defects (Argenta 1984, Radovan 1984). Silicone tissue expanders have recently been applied for expansion of blood vessels (Cohen and Ruiz-Razura 1992, Ruiz-Razura et al. 1993), viscera containing smooth muscle (Manders et al. 1987) and peripheral nerves (Manders et al. 1987, Adson et al. 1988, Anjou et al. 1989, Milner 1989, Wood et al. 1991). If a nerve is elongated by this method, then the additional length can be used to overcome a nerve defect and enable a delayed primary repair. Proximal nerve expansion and repair is comparable to conventional nerve grafting for repair of segmental defects in canine sciatic nerves as related to nerve conduction velocity and gastrocnemius contraction force (Wood et al. 1991). However, if the elongation is performed too quickly, deterioration of nerve function results (Adson et al. 1988, Anjou et al. 1989, Milner 1989, Millesi 1990). Proximal nerve expansion may have a deleterious effect on nerve repair by compressing the regenerating axons, resulting in a significant decrease in nerve conduction velocity (Milner 1989) and the histologic appearance of epineurial and endoneurial thickening (Wood et al. 1991). Distal nerve expansion involves expanding Schwann cells and their supporting elements and spares regenerating axons from expansion forces (Wood et al. 1991). To date, four clinical cases of peripheral nerve expansion have been reported with different functional results (Manders et al. 1987, van Beek 1989).

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CHAPTER 4

AIMS OF THE STUDY

Currently, peripheral nerve defects are best treated by interfascicular nerve grafting. However, nerve grafting frequently fails to achieve optimal functional results. Moreover, harvesting of the donor nerve graft is associated with the donor site-morbidity of scar, sensory loss, and occasionaly, a painful neuroma. Peripheral nerve elongation by the use of a tissue expander may offer an alternative to nerve grafting. If a nerve is elongated by this method, the additional length can be used to overcome a nerve defect and enable a delayed primary repair. Moreover, donor-site morbidity associated with nerve grafting is avoided. Nerve expansion and repair may yield better functional results since only one coaptation is involved. However, it is important to control nerve function during expansion. Nerve function and structure depend intimately on the integrity of its blood supply.

The aim of the present study is to investigate:

I laser Doppler flowmetry (LDF) as a method of monitoring nerve blood flow (NBF) during expansion.

II the impact of expansion on nerve function when NBF is controlled by LDF.

III the neurophysiological changes in peripheral nerves elongated by LDF controlled expansion.

IV the morphological changes in peripheral nerves subjected to LDF controlled expansion.

CHAPTER 5

A MODEL FOR MONITORING NERVE BLOOD FLOW DURING EXPANSION BY LASER DOPPLER FLOWMETRY IN THE RABBIT

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Summary
A new model is described for monitoring nerve blood flow during expansion by laser Doppler flowmetry. Rabbit sciatic nerve is gradually expanded with a custom-made spherical expander, while nerve blood flow is monitored by laser Doppler flow output. This model provides a valid method of controlling nerve blood flow during expansion.

Introduction

Currently, substantial loss of nerve tissue is treated by interposition of nerve grafts. However, the functional results of this technique are often disappointing and there always remain sensory loss at the donorsite and neuroma formation as a possible late complication (Millesi et al. 1972, Millesi 1990). Tissue expansion is a relative new technique to provide local tissue. Silicone tissue expanders have been used for reconstruction of skin and soft tissue defects (Van Rappard 1988). Only recently these materials have been used for expansion of blood vessels (Ruiz-Razura et al. 1989), viscera containing smooth muscle (Manders et al. 1987) and peripheral nerves (Manders et al. 1987, Anjou et al. 1989, Milner 1989, Wood et al. 1991). Nerve expansion is defined as the increase in length of a nerve by use of a tissue expander. If a nerve could be elongated by this method, the additional length could be used to overcome a nerve defect following segmental nerve loss, so that delayed primary repair with a single coaptation can be done later. In order for a nerve graft to be successful the regenerating axons must traverse two coaptations to reach the end organ. Moreover, nerve grafting necessitates the added operative time and trauma with harvesting a segment of a normal peripheral nerve (Wood et al. 1991). However, it is important to control nerve function during expansion. Nerve function depends intimately on intact nerve blood flow (NBF): while ischemia results in rapid deterioration of nerve function, nerve function recovers coincident with restoration of NBF (Lundborg and Brånemark 1968, Lundborg 1970). Laser Doppler flowmetry (LDF) can be used to examine nerve blood flow (NBF) in vivo in a linear relation between LDF and NBF (Rundquist et al. 1986, Seiler et al. 1989). The aim of the present study is to investigate whether LDF is a valid method of monitoring NBF during expansion.

Materials and Methods

The study was approved by the Committee for Animal Research of the Central Animal Laboratory of the University of Nijmegen. Ten New Zealand white rabbits weighing between 2.5 and 3.5 kg were used in this study. The animals were anesthetized with 0.5 ml/kg Hypnorm® i.m. (10 mg/ml fluanison and 0.3125 mg/ml fentanylcitrate) followed by N_2O/O_2 and ethrane inhalation. The rabbits were placed on their

left side on a thermostated heating pad that maintained rectal temperature at 36-37,5°C. The right sciatic nerve was exposed using aseptic technique. The sciatic nerve was gently placed in a specially designed teflon nerve holder. The nerve holder fits snugly in the groove of a 20 cc custom-made spherical expander (CUI Corporation Carpinteria, CA, USA) (Figures 1 and 2).

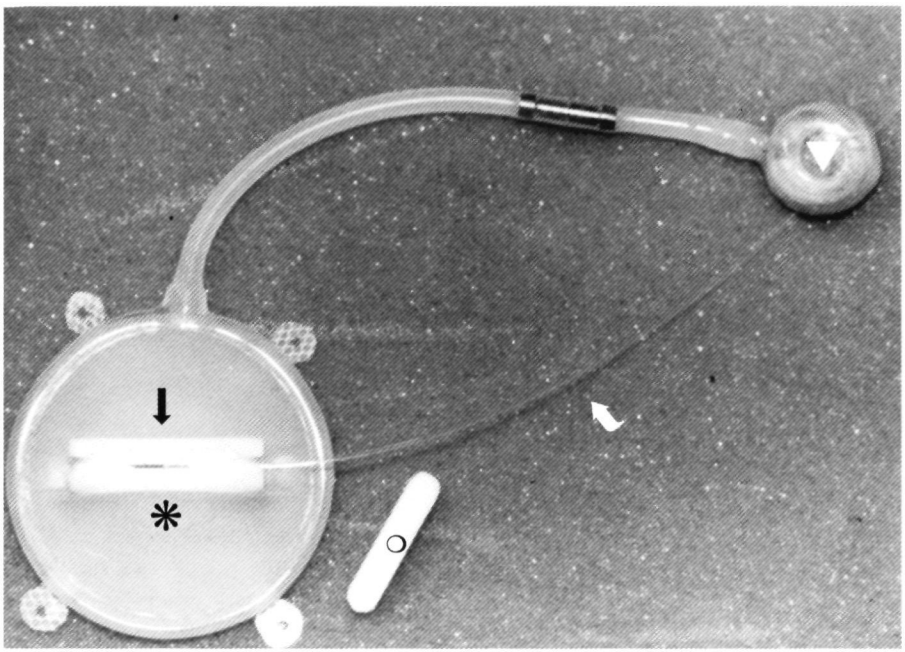

Fig. 1. The nerve expander consists of a 20 cc spherical reservoir (asterisk) with a groove for a custom-made teflon nerve holder (arrow) and fiber optics (curved arrow). The nerve holder is closed by a cover (circle) on top of it. The groove prevents luxation of the sciatic nerve and allows expansion to occur longitudinally and not radially. The expansion is performed by injection of saline into a self-sealing fill dome (triangle).

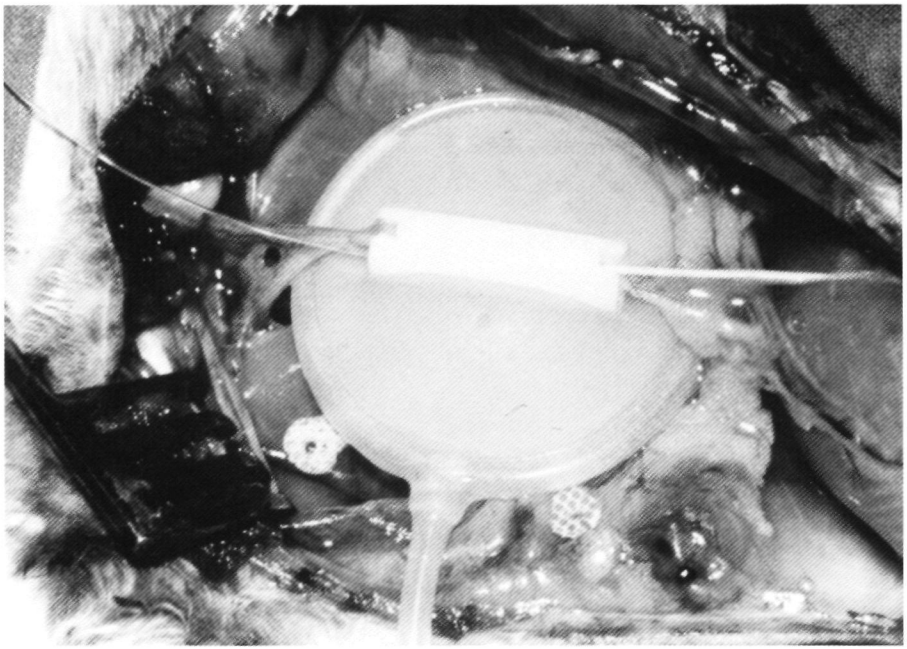

Fig. 2. Intraoperative view of the nerve-expander with the sciatic nerve and fiber optics *in situ*.

A tissue expander is effective by increasing in height through inflation of the reservoir, with only a minimal nonsignificant alteration of its longitudinal or radial dimensions. The expansion is performed by instilling saline into the self-sealing fill dome via an infusion line connected to a pressure recorder. The result is elongation of the nerve by stretching it gradually with only a minimal increase in size radially, because the nerve is fixed in the enclosed groove on top of the expander. In order to allow for a continuous recording of the NBF in a fixed point at the nerve's surface, the fiber optic probe runs in a special groove of the nerve-holder at the center of the spherical nerve expander. The LDF output was measured using an angled tip microfibre (PF 319, Perimed Co., Järfälla, Sweden) connected to a Master Probe (PF 318, Perimed Co.). The Master Probe was inserted in a laser Doppler flowmeter (Periflux 2B, Perimed Co.). The LDF signal was studied in relation to the fluid volume instilled in the tissue expander. After a base-line recording of LDF output was obtained, the expander was

inflated and the nerve gently expanded up to the level of a zero flow state. Pulsatile flow was allowed to return first by slight deflation and then complete emptying of the expander.

Results

All nerves exhibited a pulsatile flow. Small fluctuations with a frequency of 19 to 21 per minute, not synchronous with heart or respiration rate, contributed to a pulsatile steady-state LDF output. Gradual insufflation of the expander initially resulted in a decrease of the amplitude of the fluctuations without affecting LDF level. The LDF level remained constant up to a certain instilled volume and then decreased rapidly to a zero flow state. Slight deflation of the expander was sufficient for return of pulsatile flow with small amplitude. Subsequent emptying of the expander resulted in a rapid increase in amplitude and level of the LDF signal. The LDF signal reached a level superior to the steady state level after complete deflation of the expander, and gradually returned to the pre-expansion level. After release of only a small volume, the pulsatile LDF signal returned more gradually in amplitude and level up to a level inferior to the pre-expansion level. Two representative sequences of LDF changes are represented in figures 3 and 4.

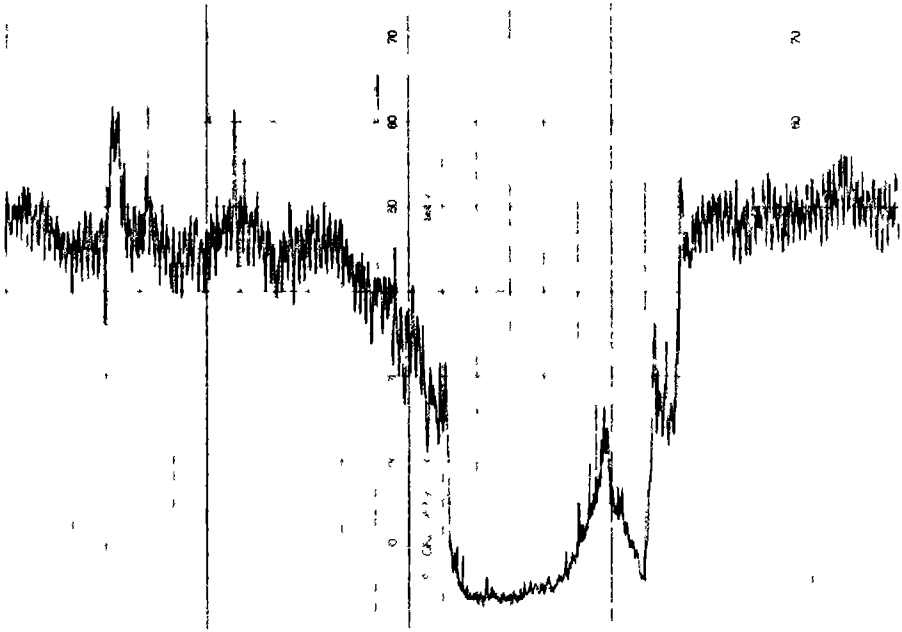

Fig. 3. Rapid return of the LDF signal in the expanded nerve after emptying the expander. The LDF output signal is expressed in perfusion units (PU). A full scale of deflection of 100 PU corresponds to 10 volts. Paper speed is three horizontal bars per minute.

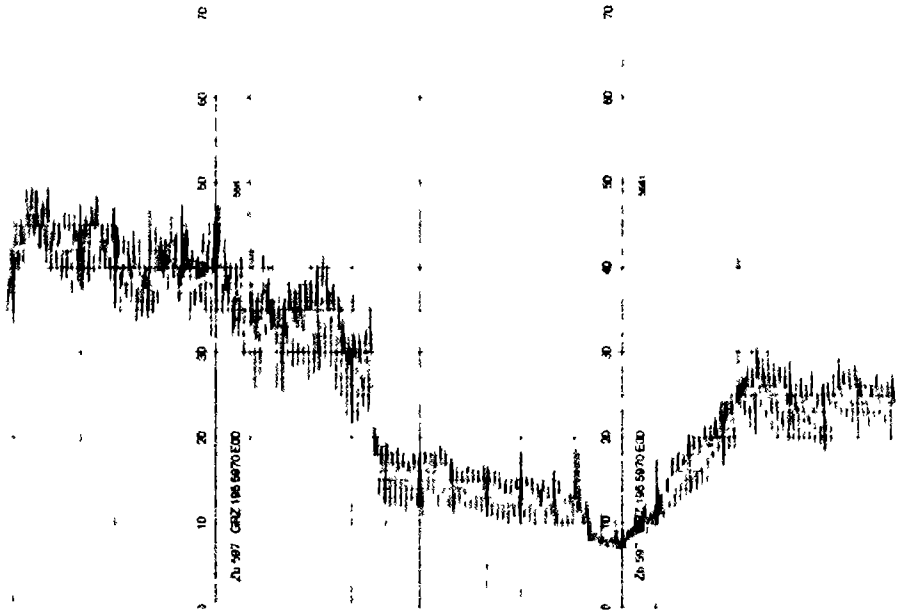

Fig. 4. A more gradual recovery of the pulsatile LDF signal after release of only a small volume out of the reservoir. The LDF output signal is expressed in perfusion units (PU). A full scale of deflection of 100 PU corresponds to 10 volts. Paper speed is three horizontal bars per minute.

Figure 3 depicts the rapid return of the LDF signal in the expanded nerve after complete emptying of the expander. Figure 4 illustrates a more gradual recovery of pulsatile flow after release of only a small volume of instilled fluid. Continuous registration of the pressure inside the filling dome revealed values not exceeding 185 mm of mercury either in the open wound or after closure of the wound. The highest levels of intra-expander pressure were associated with a zero flow state for a few minutes at most, and returned instantaneous to levels of 10 to 20 mm of mercury concomitant with slight deflation of the expander and return of the pulsatile LDF signal (data not shown).

Discussion

The blood flow to peripheral nerves consists of an extrinsic circulation of arterioles and venules in the epineural space and an intrinsic plexus of capillaries and other microvessels within the endoneurium. The two circulations are connected by anastomotic microvessels traversing the perineurium. The direction of blood flow in individual vessels within the collateral system is not uniform and occasionally may be even reversed (Lundborg and Brånemark 1968, Lundborg 1975).

Although peripheral nerves are relatively resistent to the effects of ischemia, nerves may be injured by a profound interruption of blood flow (Rechthand et al. 1988). Nerve function and structure depend intimately on the integrity of NBF (Lundborg and Brånemark 1968, Lundborg 1970).

The present study involves insertion of a tissue expander after mobilization of the sciatic nerve, and subsequent elongation of the nerve by insufflation of the expander. While mobilization may affect the extrinsic blood supply to peripheral nerves, stretching or elongation of the nerve primarily influences the intrinsic vascular supply (Lundborg and Rydevik 1973). Mobilization of the sciatic-tibial nerve of the rabbit even over a length of 15 cm has little or no effect on the nutritive intrafascicular microcirculation on microscopic examination (Lundborg 1970). However, surgical dissection of the proximal one-third of the rabbit sciatic nerve decreased blood flow rate to an average of 22 percent and dissection of the popliteal portion caused a decrease of 17 percent of the control value, using the hydrogen washout technique. The blood flow rate to the mid-thigh portion of the sciatic nerve was not influenced by the procedure (Ogata and Naito 1986). At 8 percent elongation of the tibial nerve of the rabbit, a definite reduction in more than 50 percent of the observed venules occurs, and at 15 percent elongation the flow in all intraneural vessels ceases entirely on morphological visual observation (Lundborg and Rydevik 1973). These values of critical stretching on the intraneural blood flow correspond well with the 15.7 percent of critical elongation using the hydrogen washout technique (Ogata and Naito 1986).

In the present study, pressure on the nerve was established by gently inflating a tissue expander and reached for a few minutes values up to 185 mm of mercury measured inside the filling dome. The peak value corresponds as to its effect to the cuff pressure of about 80 mm of mercury necessary to stop intraneural circulation (Rydevik et al. 1981).

Compression of the rabbit tibial nerve at 50 mm of mercury by a cuff around the leg for two hours induced an epineurial oedema (Rydevik and Lundborg 1977). The duration as well as the magnitude of the pressure on a nerve may influence its structure and function. The effect of pressure on a nerve has been related to secondary ischemia by the occlusion of small vessels (Denny-Brown and Brenner 1944) and to a pressure gradient in the nerve between its compressed and uncompressed parts (Ochoa et al. 1972, Gilliatt et al. 1974).

NBF has been determined with the hydrogen clearance technique in cats (Smith et al. 1977) and with [14C]iodoantipyrine in rats (Myers et al. 1982), but these methods have a limited time resolution. LDF is a non-invasive technique that allows continuous recording, measures microvascular blood flow directly and provides an easily interpretable numerical output with a short response time (Svensson 1986). The LDF output signal is influenced by probe motion and position, bleeding, serum percent oxygen saturation, serum hematocrit levels and triglyceride content (Nilsson et al. 1980, Swiontkowski et al. 1986). LDF has been shown to be useful as a monitoring device in assessing skin perfusion and the viability of transferred free flaps (Svensson 1986). The output signal is expressed in volts, which is not a unit of flow, but the output signal has been shown to correlate highly with blood flow. The LDF signal from the sciatic nerve of the anesthesized rat is linearly related to NBF as measured by [14C]iodoantipyrine (Rundquist et al. 1985). Release of the transverse carpal ligament resulted in the return of pulsatile LDF signal within 1 minute in median nerves of patients with clinical carpal tunnel syndrome (Seiler et al. 1989).

The present study is the first investigation that has addressed itself to monitoring nerve blood flow during nerve expansion. Sciatic NBF was examined by LDF in anesthesized rabbits during nerve expansion after insertion of a custom-made tissue expander. NBF displayed a pulsatile signal after insertion of the expander. The small fluctuations in the LDF signal with a frequency of 19 to 21 per minute were not synchronous with heart or respiration rate. The origin and functional importance of these rhythmic fluctuations are not completely understood, but the oscillations may have been due to spontaneous rhythmic contractions of arteriolar smooth muscle of the kind previously demonstrated in human skin and rat testis (Tenland 1982). Gradual

insufflation of the expander resulted in a diminution of amplitude and level of NBF. At a critical point NBF reacted with a sudden decrease up to a zero flow state. NBF returned to a pulsatile flow state within seconds after release of a small volume of instilled fluid.

This study shows that laser Doppler flowmetry is a valid method of monitoring nerve blood flow during expansion. This method may prove useful in monitoring nerve function indirectly during nerve expansion. Future studies are needed to elucidate the relation between intact nerve blood flow during sciatic nerve expansion and postoperative function of the rabbit hind leg.

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CHAPTER 6

PERIPHERAL NERVE ELONGATION BY LASER DOPPLER FLOWMETRY CONTROLLED EXPANSION: FUNCTIONAL AND NEUROPHYSIOLOGICAL ASPECTS

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Summary
A new method for elongation of peripheral nerves with preservation of function is presented. Nerve blood flow during experimental nerve expansion of rabbit sciatic nerve is controlled by laser Doppler flowmetry in order to avoid nerve ischemia. Using this method, nerve function in relation to gait remained intact in 72.5 % of the animals and recovered within three weeks in the others. Disturbances in toe-spread reflex recovered completely in all animals after three weeks. Significant nerve elongation up to 40% is possible with preservation of function when nerve blood flow is controlled by laser Doppler flowmetry.

Introduction

Currently, substantial peripheral nerve defects are treated by interposition of a nerve graft. In order for a nerve graft to be successful the regenerating axons must traverse two coaptations to reach the end organ. The functional results of this technique are often disappointing and there is always sensory loss at the donor site with neuroma formation as a possible late complication (Millesi et al. 1972, Millesi 1990). Moreover, nerve grafting necessitates the added operating time and trauma associated with harvesting a segment of a normal nerve (Wood et al. 1991). Tissue expansion is a relatively new technique for providing local tissue. Tissue expanders have recently been used for the expansion of blood vessels (Cohen and Ruiz-Razura 1992), viscera containing smooth muscle (Manders et al. 1987) and peripheral nerves (Manders et al. 1987, Anjou et al. 1989, Milner 1989, Wood et al. 1991). If a nerve could be elongated by the use of a tissue expander, the additional length could be used to overcome a nerve defect, so that delayed primary repair with a single coaptation can be done later. In addition, donor site morbidity associated with nerve grafting is avoided. The functional outcome of this method may prove to be superior to nerve grafting as only one nerve anastomosis is involved. However, it is important to control nerve function during expansion. Nerve function depends intimately on an intact nerve blood flow (NBF): nerve function rapidly deteriorates as a result of ischemia but recovers with restoration of NBF (Lundborg and Brånemark 1968, Lundborg 1970). Laser Doppler flowmetry (LDF) can be used to examine NBF *in vivo*, as a linear relation exists (Rundquist et al. 1986, Seiler et al. 1989). LDF is a valid method of monitoring NBF, and allows avoidance of nerve ischemia during nerve expansion (van der Wey et al. 1993). The aim of the present study is to determine whether a nerve can be elongated by a tissue expander with preservation of function.

Materials and methods

General

The study was approved by the Committee for Animal Research of the Central Animal Laboratory of the University of Nijmegen. Fifty adult New Zealand white rabbits weighing between 2.5 and 3.5 kg were

used in this study. The rabbits were housed in individual cages and received small animal diet and water *ad libitum*. The animals were divided at random in a sham group (n=11) and an experimental group (n=39). The rabbits in the experimental group were randomly assigned to three subgroups, in relation to the instilled expander volume: 5 cc (n=12), 10 cc (n=13) or 15 cc (n=14). Ten animals were withdrawn from the study following anesthetic death (n=2), pneumonia (n=3), expander failure (n=4) or wound infection (n=1). Thus, 40 rabbits were available in four groups: sham (n=10), 5 cc (n=10), 10 cc (n=11) and 15 cc (n=9) (Table 1).

Table 1: Complications. 50 animals had tissue expanders inserted; early complications occurred in 10 animals which led to their removal from the study; 40 animals completed expansion successfully.

Animals withdrawn			**10**
wound infection			1
damaged expander			4
pneumonia			3
anesthetic death			2
Successful expansion			**40**
	2 weeks	6 weeks	
sham	4	6	10
5 cc	5	5	10
10 cc	7	4	11
15 cc	4	5	9

Operation

The animals were anesthetized using 0.5 ml/kg Hypnorm® i.m. (10 mg/ml fluanison and 0.3125 mg/ml fentanylcitrate) followed by N_2O/O_2 and ethrane inhalation. The rabbits were placed on their left side on a thermostated heating pad that maintained rectal temperature at 36-37,5°C. The right sciatic nerve was exposed using aseptic technique. Four 9/0 Ethilon® sutures were placed in the epineurium at distances of 10 mm. The sciatic nerve was gently placed in a specially

designed Teflon nerve holder. The nerve holder fits snugly in a 20 cc custom-made spherical expander (CUI Corporation Carpinteria, CA, USA). The tissue expander and the procedure for LDF registration have been described before by van der Wey et al. (1993). A tissue expander is effective by increasing in height through inflation of the reservoir, with only a minimal nonsignificant alteration of its other dimensions. The result is elongation of the nerve by stretching it gradually with only a minimal increase in size radially, because the nerve is fixed in the enclosed groove on top of the expander (Figure 1).

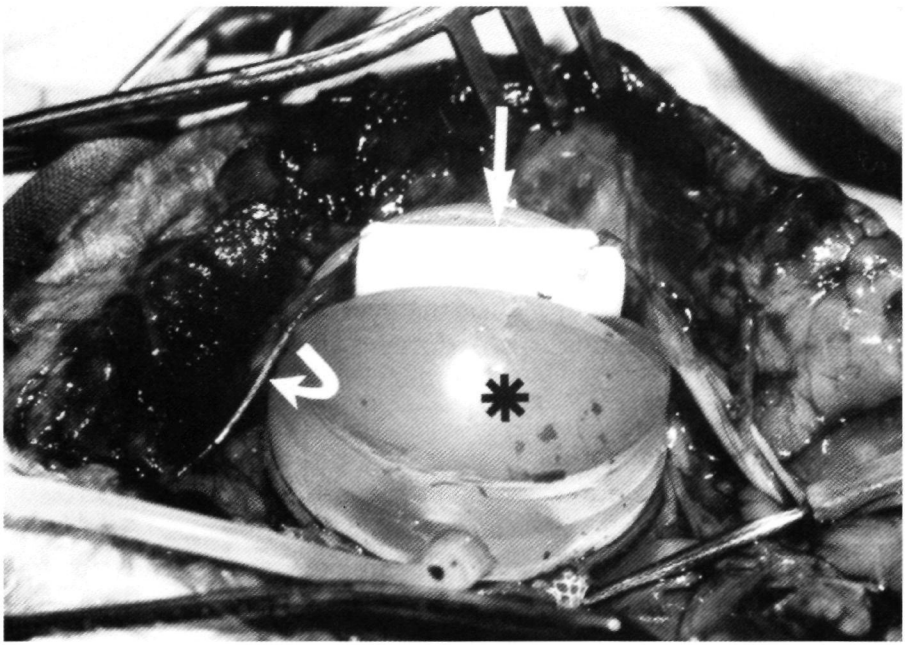

Fig. 1. Intraoperative view of the fully inflated nerve-expander (asterisk) with the sciatic nerve contained within the Teflon nerve holder (large arrow) and fiber optics (curved arrow) in situ.

In order to facilitate a continuous recording of NBF at a fixed point on the nerve's surface, the fiber optic probe runs in a special groove in the nerve-holder at the center of the spherical nerve expander. The LDF output was measured in the experimental group using an angled tip microfibre (PF 319, Perimed Corp., Järfälla, Sweden) connected to a

Master Probe (PF 318, Perimed Corp.). The Master Probe was inserted in a laser Doppler flowmeter (Periflux 2B, Perimed Corp.). After a base-line recording of pulsatile LDF output was obtained, the microfibre was disconnected. A subcutaneous tunnel on the animal's back accommodated the fiber optics and the fill-dome. The rabbits were daily examined postoperatively in relation to gait and toe-spread reflex (TSR). Gait was scored as normal or impaired due to paresis or plegia of the operated leg. TSR was scored as normal, decreased or absent. The animals in the experimental group were re-operated for LDF controlled expansion of the sciatic nerve at weekly intervals. A base-line recording of LDF output was obtained after exposure of fiber optics and fill-dome. The expander was gradually inflated with 2 to 4 cc of warm saline (approximately 30°C) while pulsatile LDF was preserved. This procedure was repeated at weekly intervals until the desired expander volume of 5, 10 or 15 cc was obtained. The expander was left fully inflated for two or six weeks. All animals were re-operated two or six weeks following either implantation of the nerve-expander (sham group) or completion of the desired expander volume of 5, 10 or 15 cc (experimental group). Measurement of nerve elongation and neurophysiological testing was performed after removal of the deflated expander.

Measurement of elongation

Nerve expansion is defined as the increase in length of the nerve by use of a tissue expander. Nerve elongation was measured by adding up the distances between the epineurial marker sutures. The degree of elongation is the increase in length of the nerve following removal of the expander as compared with the original length expressed as a percentage.

Neurophysiological methods

The temperature of the nerves was controlled by bathing the relevant section in a saline solution of approximately 30°C. The positions of the stimulating and recording electrodes in relation to the expanded region of the sciatic nerve are clarified in Figure 2.

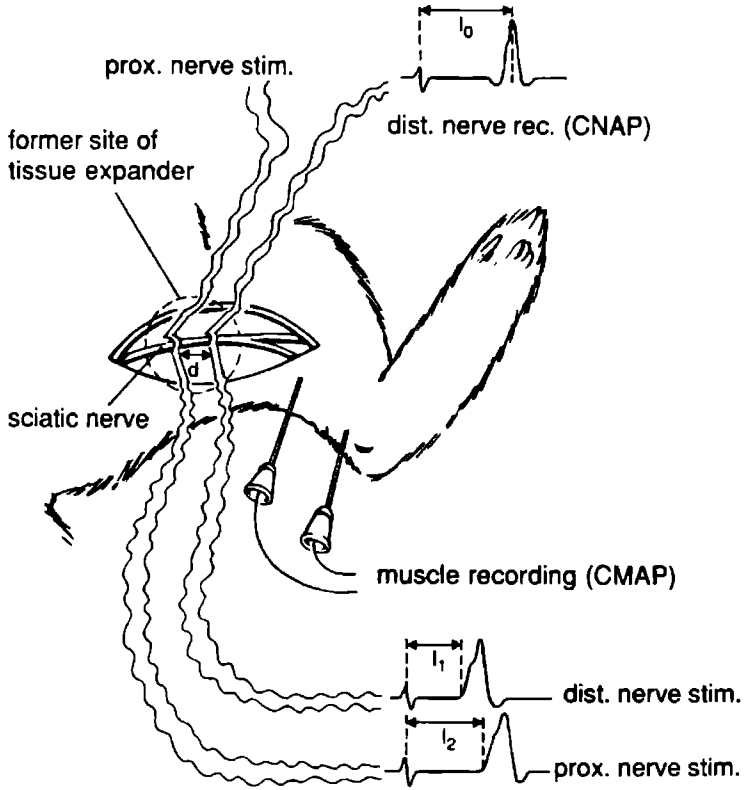

Fig. 2. Diagram of neurophysiological studies. The electrodes are applied to the expanded region of the sciatic nerve after removal of the expander.
CNAP: conduction velocity = d/L_0;
CMAP: conduction velocity = d/L_2-L_1.

A stimulating electrode consisting of two stainless steel hooks, approximately 1.5 mm apart, was gently applied to the nerve in the proximal part of the expanded region. A rectangular monophasic current pulse (duration 0.2 ms) was passed through this electrode. The relatively long stimulus pulse duration was chosen to ensure optimal stimulation of the nerve without the large stimulus spread caused by a higher stimulus current level in connection with shorter pulse durations. The stimulating voltage was set at three times the threshold voltage required to produce an action potential, amply beyond the supramaximal level at which the whole nerve fiber population is activated

(Gorman and Mortimer 1983). A similar electrode was applied 18 mm distal to the first electrode for registration of the compound nerve action potential (CNAP). CNAP was quantified by its main peak latency and peak-to-peak amplitude. Combined with the interelectrode distance the nerve conduction velocity (NCV) was assessed. Compound muscle action potentials (CMAPs) were measured in the gastrocnemius muscle with two monopolar needle electrodes following stimulation of the sciatic nerve by the proximal and the distal electrode respectively. The NCV was calculated from the latency difference between the initial deflections of the two responses and the interelectrode distance. The same procedure was performed on the contralateral nerve as a control.

Statistical methods

A test for normal distribution suggested that the results were normally distributed. Data in each group are presented as mean values ± SEM. Multiple linear regression calculations and analysis of variance were performed on the relation of nerve elongation versus expander volume, and on the neurophysiological data of the expanded nerve and its control. In all statistical analysis $P<0.05$ was considered as being statistically significant.

Results

Nerve elongation

The data of nerve elongation are presented in Table 2 and Figure 3. The degree of elongation ranged from 10.0 ± 0% to 38.6 ± 5.6% (Table 2).

Table 2: Degree of elongation (%) in relation to instilled expander volume.

	2 weeks	6 weeks
sham	10.0 ± 0	20.0 ± 2.7
5 cc	22.0 ± 1.8	21.7 ± 2.2
10 cc	26.7 ± 1.9	36.3 ± 4.3
15 cc	34.2 ± 2.7	38.6 ± 5.6

Results are expressed as mean values ± SEM

Insertion of the nerve expander resulted in an elongation of 10.0 ± 0% after two weeks, and 20.0 ± 2.7% after six weeks. A highly significant trend existed between nerve elongation and instilled expander volume (1.5%/cc, $p<0.001$). The expander produced a significant additional increase in length of the nerve of 5.4% between two and six weeks ($p<0.05$) (Figure 3).

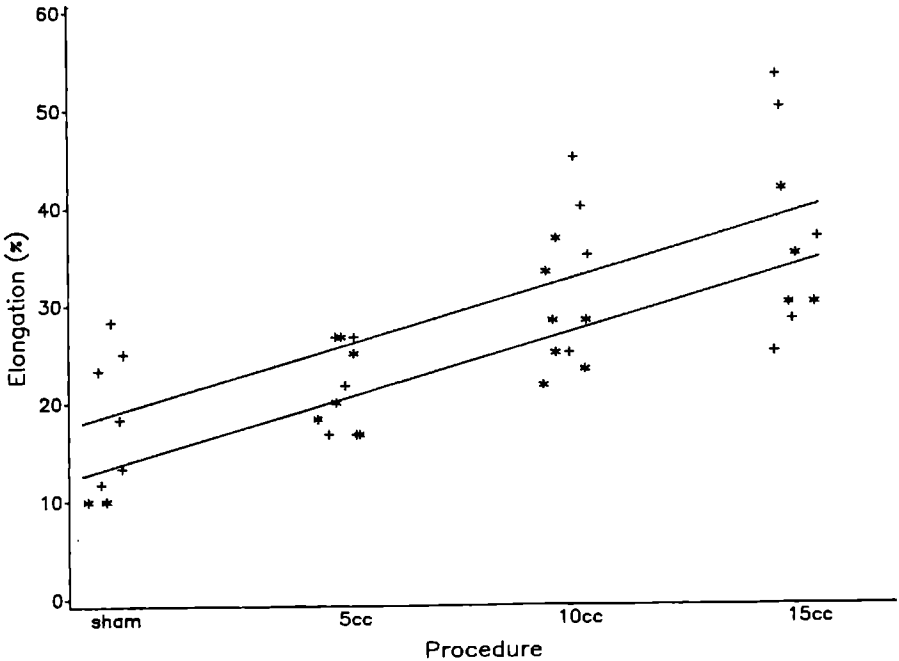

Fig. 3. Degree of elongation in relation to instilled expander volume. Symbols: *: Restperiod of two weeks; +: Restperiod of six weeks.

Functional assessment

Any disturbances in gait or TSR were observed initially after insertion of the expander and did not present in the course of the expansion procedure. Gait remained normal in 29 rabbits (72.5%). Eleven rabbits (27.5 %) had a paresis of the operated leg for an average of 19.4 ± 3.2 days. TSR was decreased in 23 (57.5 %) and absent in 17 rabbits (42.5 %). TSR recovered completely in all animals after an average period of 22 ± 3.3 days. Time to full recovery of gait or TSR was not significantly related to the instilled expander volume (data not shown).

CNAP

The data are presented in Tables 3 and 5. The sham procedure resulted in a highly significant decrease of NCV ($p<0.001$) and increase of threshold ($p<0.001$), and a significant decrease of amplitude ($p<0.005$). NCV decreased highly significantly in relation to instilled volume (1.8%/cc, $p<0.001$) and significantly in relation to elongation (0.8% per percent elongation, $p<0.01$). Amplitude and threshold did not change in relation to instilled volume or elongation. NCV, amplitude and threshold did not change from two to six weeks.

Table 3: Compound nerve action potential.

NCV (m/sec)

	weeks	expander	control
sham	2	30.7±3.2	44.4±6.0
	6	20.8±10.7	37.1±2.1
5 cc	2	31.4±3.3	41.8±2.8
	6	37.5±3.6	48.1±1.3
10 cc	2	28.3±2.0	42.0±1.6
	6	26.2±2.0	46.5±1.3
15 cc	2	21.1±1.6	44.6±4.0
	6	34.7±13.3	69.9±3.0

Threshold (mA)

	weeks	expander	control
sham	2	0.7±0.3	0.2±0.1
	6	0.4±0.0	0.1±0.0
5 cc	2	0.9±0.3	0.3±0.1
	6	0.6±0.1	0.4±0.1
10 cc	2	1.2±0.2	0.3±0.1
	6	1.4±0.4	0.9±0.3
15 cc	2	1.7±0.4	0.4±0.2
	6	1.6±0.6	0.2±0.0

Amplitude (mV)

	weeks	expander	control
sham	2	0.3±0.2	0.9±0.4
	6	1.5±0.6	0.3±0.1
5 cc	2	2.6±0.4	0.9±0.2
	6	1.3±0.4	1.2±0.3
10 cc	2	2.1±0.3	0.7±0.2
	6	1.0±0.3	0.5±0.2
15 cc	2	1.9±1.0	0.6±0.2
	6	0.5±0.2	1.8±0.4

Results: mean ± SEM

CMAP

The data are presented in Tables 4 and 5. The sham procedure resulted in a significant decrease of NCV ($p<0.05$) and a highly significant increase of threshold ($p<0.001$), but did not change amplitude. NCV did not change in relation to instilled volume, but decreased significantly in relation to elongation (1.4% per percent elongation, $p<0.01$). Amplitude and threshold did not change in relation to instilled volume or elongation. NCV, amplitude and threshold did not change from two to six weeks.

Table 4: Compound muscle action potential.

NCV (m/sec)

	weeks	expander	control
sham	2	46.5±5.9	62.6±7.6
	6	54.4±16.3	53.1±6.2
5 cc	2	53.1±8.5	59.5±12.7
	6	61.7±4.7	75.1±10.5
10 cc	2	43.3±8.8	67.8±7.6
	6	54.6±18.1	93.8±12.2
15 cc	2	25.7±14.7	47.7±7.7
	6	34.7±13.3	69.9±3.0

Threshold (mA)

	weeks	expander	control
sham	2	0.8±0.2	0.3±0.1
	6	0.6±0.2	0.2±0.1
5 cc	2	1.0±0.3	0.2±0.1
	6	1.0±0.3	0.3±0.1
10 cc	2	1.1±0.2	0.3±0.0
	6	1.6±0.5	0.6±0.3
15 cc	2	2.4±0.7	0.5±0.2
	6	1.6±0.6	0.2±0.1

Amplitude (mV)

	weeks	expander	control
sham	2	24.5±5.7	27.5±4.4
	6	15.0±2.8	21.4±6.3
5 cc	2	25.1±2.8	29.2±6.4
	6	27.8±4.0	44.3±4.3
10 cc	2	28.8±6.4	32.2±6.5
	6	29.5±3.0	42.8±0.7
15 cc	2	12.2±5.5	25.5±10.2
	6	20.9±8.0	34.8±1.1

Results: mean ± SEM

Table 5: NCV, amplitude and threshold in relation to instilled expander volume and degree of elongation.

Compound nerve action potential

		endvolume	elongation
	sham value (%)	slope (%/cc)	slope (%/%)
NCV	82****	-1.8****	-0.8**
Amplitude	42***	0.8^{ns}	-0.5^{ns}
Threshold	48****	-0.8^{ns}	-0.7^{ns}

Compound muscle action potential

	endvolume	elongation
	sham value (%)	slope (%/cc)	slope (%/%)
NCV	81*	-1.5ns	-1.4**
Amplitude	101ns	0.6ns	-1.9ns
Threshold	45****	-1.5ns	-0.9ns

Values represent the ratio of expander and control values (NCV, amplitude) or the ratio of control and expander values (threshold) times 100%. Symbols: *: P<0.05; **: P<0.01; ***: P<0.005; ****: P<0.001; ns: not significant.

Discussion

The present study revealed that it is possible to elongate rabbit sciatic nerve up to 40% by means of a tissue expander. All experiments were performed in adult rabbits, excluding growth of the rabbit as a factor in nerve elongation. The effect of the uninflated expander in place is illustrated in a nerve elongation of 10% after two weeks and 20% after six weeks in the sham group. The increase in length of the nerve was linearly related to the volume injected in the tissue expander. The additional elongation from two to six weeks at a given expander volume, stressed the effect of the duration of nerve expansion. Sciatic nerve function remained intact during expansion in the majority of animals. The rapid recovery of any disturbances in gait or TSR at the end of the third week is indicative of a first degree nerve lesion (Sunderland 1978). In contrast, NCV decreased in a linear relation to elongation and did not recover.

The nerve is subjected to two different kinds of trauma during expansion: stretch and compression. Nerve stretching adversely affects NBF. An elongation of 15% of rabbit sciatic nerve has previously been considered as critical as it results in ischemia (Lundborg and Rydevik 1973, Ogata and Naito 1986). Thinner myelinated fibers and non-myelinated fibers are more vulnerable to lack of oxygen than are thicker ones (Lundborg 1970, Dahlin et al. 1989). In addition, ischemic sensitivity may be related to the nutritional supply and metabolic demands of the

various fiber types (Lundborg 1970) rather than axon diameter (Mäkitie and Teräväinen 1977, Nitz et al. 1989). Nerve function depends on intact nerve vascularisation (Lundborg and Brånemark 1968, Lundborg 1970). Compromise of the intrinsic nerve circulation always results in sciatic nerve deficit (Nitz et al. 1986). The intrinsic circulation of the sciatic nerve is not influenced by mobilisation of the nerve (Lundborg 1970). The extrinsic circulation remains intact in our model, in view of the preservation of the small fluctuations in the LDF signal that are indicative of arteriolar contractions in the extrinsic circulation (van der Wey et al. 1993). Thus, LDF controlled expansion preserves NBF but may still have a certain pressure effect on the nerve.

Local pressure may result in an acute deterioration of nerve function. The effect of compression on a nerve has been related to secondary ischemia by the occlusion of small vessels (Denny-Brown and Brenner 1944) and to a pressure gradient in the nerve between its compressed and uncompressed parts resulting in displacement of the nodes of Ranvier (Ochoa et al. 1972, Gilliatt et al. 1974). The fast-conducting large-diameter nerve fibers are more susceptible to compression than thinner ones, probably because of greater deformation at a given pressure (McGregor et al. 1975). Both the level of pressure and the duration of pressure are important factors (Lundborg 1988). In our experiment, the tissue expander was inserted beneath the nerve and left in place, resulting in a permanent pressure effect. The local pressure at the sciatic nerve has not been measured, but probably can be assessed by inference. The preservation of NBF in the expanded nerve signifies that pressure at the nerve was lower than 80 mm Hg, because a local pressure of 60 to 80 mm Hg causes complete standstill of intraneural blood flow (Ogata and Naito 1986, Rydevik et al. 1981). Relatively low pressure levels of 20-30 mm Hg applied for 8 hours induce a significant block in nerve conduction (Dahlin et al. 1989). The duration of recovery of paresis and TSR in our experiments concurred with the effect of 400 mm Hg tourniquet pressure for 1 hour (Nitz et al. 1986). The temporary deterioration in function is probably a result of a local pressure gradient rather than ischemia.

The NCV decreased in relation to elongation. Elongation of a nerve results in a decrease in axon diameter (Sunderland 1978). The conduction velocity of myelinated nerve fibers is related to the diameter of the nerve fiber (Gasser and Erlanger 1929, Waxman 1980). The relation

between external fiber diameter (axon and myelin sheath) and NCV is approximately proportional (Hursh 1939, Gasser and Grundfest 1939). The permanent decrease in NCV in our study may reflect the elongation and concomitant 'thinning out' of the expanded axons rather than a morphological lesion, in view of intact sciatic nerve function in all rabbits at the end of the third week. In addition, decreased NCV may be secondary to an increase of internodal distance, abnormalities in myelin impedance or membrane permeability without loss of axonal continuity (Ochoa et al. 1972). The CNAP amplitude is severely reduced, and the threshold both for CNAP and CMAP stimulation is increased after implantation of the expander. A decreased CNAP amplitude can be secondary to a relatively mild general nerve velocity decrease without any nerve fiber blocking (Swenson and Cornacchia 1991). A similar NCV mediated amplitude effect is only marginally expected for CMAP. Neither is an increased threshold to stimulation necessarily related to a severe conduction block. In conclusion, the neurophysiological parameters seem to be impaired without blocking, which is in concurrence with the normal or rapidly normalizing sciatic nerve function of the animals.

Nerve expansion results in a significant decrease in conduction velocity of expanded nerves (Milner 1989). Controlled expansion and repair of canine sciatic nerve resulted in neurophysiological and functional results comparable to nerve grafting after 18 months (Wood et al. 1991). The present experiment shows, that significant nerve elongation up to 40% is possible with preservation of function when NBF is controlled by LDF. The temporary loss of gait and TSR in a minority of rabbits, indicative of a first degree nerve lesion according to Sunderland, is apparently related to a local pressure effect on the nerve.

Monitoring nerve blood flow during expansion is important because nerve function depends intimately on the integrity of NBF (Lundborg and Brånemark 1968, Lundborg 1970). Laser Doppler flowmetry is a valid method of monitoring nerve blood flow during expansion (van der Wey et al. 1993). The temporary functional deterioration in a minority of the animals seems acceptable in view of the substantial elongation of the sciatic nerve. Monitoring nerve blood flow during expansion appears as a significant factor for postoperative function of the rabbit hind leg.

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CHAPTER 7

PERIPHERAL NERVE ELONGATION BY LASER DOPPLER FLOWMETRY CONTROLLED EXPANSION: MORPHOLOGICAL ASPECTS

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Abstract
Peripheral nerve elongation by a tissue expander may offer an alternative to nerve grafting in the management of segmental nerve loss. We investigated the morphological changes in peripheral nerve following slow nerve elongation by laser Doppler flowmetry controlled expansion in a rabbit sciatic nerve model. The animals were randomly assigned to four groups with an expander volume of 0, 5, 10 or 15 cm^3. Elongation was possible up to 40% with preservation of clinical function. Nerve conduction velocity decreased in relation to elongation. Paranodal widening, followed by remyelination of the node were early and constant morphologic features. Demyelination and remyelination of whole internodes, and axonal degeneration occurred sporadically and did not correlate with elongation, rate of elongation or neurophysiological parameters. The model of laser Doppler flowmetry controlled nerve expansion provides for remodelling of myelin sheaths and lengthening of nerve fibers without axonal damage.

Introduction

Tissue expansion was introduced into clinical practice in 1957 [15]. Silicone tissue expanders have been widely used to provide local tissue for reconstruction of skin and soft tissue defects [1, 20]. These materials have recently been applied for expansion of peripheral nerves [11, 13, 28]. If a nerve is elongated by this method, the additional length can be used to overcome a nerve defect and enable a delayed primary repair with a single coaptation. However, it is important to control nerve function during expansion. Nerve function and structure depend intimately on the integrity of its blood supply [9, 10]. Laser Doppler flowmetry (LDF) has been established as a valid method of monitoring nerve blood flow (NBF) during nerve expansion in order to avoid ischemia [26]. Using this technique significant elongation of rabbit sciatic nerve up to 40% was possible with preservation of clinical function [27]. Nerve conduction velocity (NCV) decreased with increasing elongation without blocking of nerve fiber impulses [27]. The present study reports an analysis of morphological changes in peripheral nerves elongated by LDF controlled expansion.

Materials and methods

General

The study was approved by the Committee for Animal Research of the Central Animal Laboratory of the University of Nijmegen. Fifty adult New Zealand white rabbits weighing between 2.5 and 3.5 kg were used in this study. The rabbits were housed in individual cages and received small-animal diet and water *ad libitum*. The animals were randomly assigned to four groups: 0 cm^3 (n=11), 5 cm^3 (n=12), 10 cm^3 (n=13) or 15 cm^3 (n=14). Ten animals were withdrawn from the study following anesthetic death (n=2), pneumonia (n=3), expander failure (n=4) or wound infection (n=1). Thus, 40 rabbits were available in four groups: 0 cm^3 (n=10), 5 cm^3 (n=10), 10 cm^3 (n=11) and 15 cm^3 (n=9).

Operation

The procedure for insertion of the nerve expander and LDF measurement has been described before [26, 27]. A nerve expander is effective by increasing in height through inflation of the reservoir, with

only a minimal nonsignificant alteration of its longitudinal or radial dimensions. The expansion is performed by instilling saline into the self-sealing fill-dome. The result is elongation of the nerve by stretching it gradually with only a minimal increase in size radially, because the nerve is fixed in the enclosed groove on top of the expander [27]. The animals were re-operated for LDF controlled expansion. A baseline recording of LDF output was obtained after exposure of fiber optics and fill-dome. The expander was gradually inflated with 2 to 4 cm^3 of warm saline (approximately 30°C) while pulsatile LDF was preserved. This procedure was repeated at weekly intervals until the desired expander volume of 5, 10 or 15 cm^3 was obtained. The expander was left fully inflated for two or six weeks. The animals were re-operated two or six weeks following either implantation of the expander (0 cm^3) or completion of the desired expander volume of 5, 10 or 15 cm^3.

Morphological techniques

The animals were sacrificed for morphological investigation after measurement of nerve elongation and neurophysiology studies [27]. The sciatic nerve was removed immediately. Three parts of the nerve were processed for microscopy studies: one part proximal to the expander, a second part in the middle of the region overlying the expander, and a third part distal to the expander. The other sciatic nerve was used as a control. Small nerve fragments of each part were fixed in 2% glutaraldehyde buffered with sodium cacodylate pH 7.4, postfixed in 2% osmiumtetroxide in Palade buffer pH 7.4 and after dehydration in alcohol, embedded in Epon 812. Semithin 1-μm-thick transverse sections were stained with 1% toluidine blue. The transverse area of each fascicle (TFA_j) and the total transverse fascicular area (TTFA) were determined with the aid of a planimeter on projected enlargements of semithin transverse sections of the whole nerve. The presence of six morphological characteristics (i) in each fascicle (j) was classified lightmicroscopically following a five point scale ranging from "not present" ($s_{ij} = 0$) to "marked" ($s_{ij} = 4$). The following parameters were assessed: axonal degeneration, cluster formation, demyelination (paranodal or segmental loss of myelin), remyelination (axons with disproportionately thin myelin), redundant myelin loop formation and endoneurial edema. For each parameter, the contribution of

the fascicle was determined by multiplying the grade of severity (s_{ij} = 0-4) and the fraction of the TTFA occupied by the fascicle j (TFA_j / TTFA). The "total" grade of pathology for each morphological characteristic (G_i) was obtained by the sum of the values of each fascicle. Thus,

$$G_i = \sum_{j=1}^{n} \frac{TFA_j}{TTFA} . s_{ij}$$

Like s_{ij}, G_i ranges between 0-4. Part of the nerve directly distal to the expander was fixed in 2% calciumchloride and 4% formaldehyde, and postfixed in 1% osmiumtetroxide in 0.1 M phosphate buffer pH 7.4. Single myelinated fibers were teased and classified according to Dyck [3]. Internodal (segment) length was determined in 100 teased fibers of expanded nerves and control nerves, and plotted against fiber diameter. The internodal length was classified as to the length of the myelinated part of the internode (IL_m) and the length of the whole internode including the paranodal area (IL_{sum}). The total number of degenerating fibers was counted in the distal part of the expanded nerves. Fiber densities and diameter distributions of myelinated axons were determined from electron microscopic photographs (1500 ×) of ultrathin sections of the expanded nerve and of the control nerve of six animals using a Zeiss TGZ 3 particle size analyzer.

Statistical methods

Pearson correlation, paired and unpaired Student's T-statistics were performed on the morphological data of the expanded nerve and its control versus the degree and rate of elongation, NCV and amplitude of CNAP. Multiple linear regression calculations and analysis of variance were performed on the relation of IL_m and IL_{sum} versus fiber diameter in expanded nerves and control nerves. In all statistical analysis $P<0.05$ was considered as significant.

Results

Transverse sections

Edema was present at all levels. The part proximal to the expander did not show any morphological changes except edema. Axons without

myelin and thinly (re)myelinated axons occurred in the middle and distal parts (Fig.1). Degeneration of axons occurred sporadically in these parts. Axons with dark cytoplasma typical for ischemia were not observed [8, 16]. Several single, apparently regenerated fibers occurred within Schwann cells still loaded with myelin debris. Groups of small regenerated axons (clusters) were seen occasionally. The Schmidt-Lanterman incisures were dilated at all levels. Signs of chronic nerve compression (e.g. redundant myelin loop formation) were seen sporadically at the middle and distal parts. The perineurium showed no abnormalities, whereas epineurial thickening occurred in the middle part.

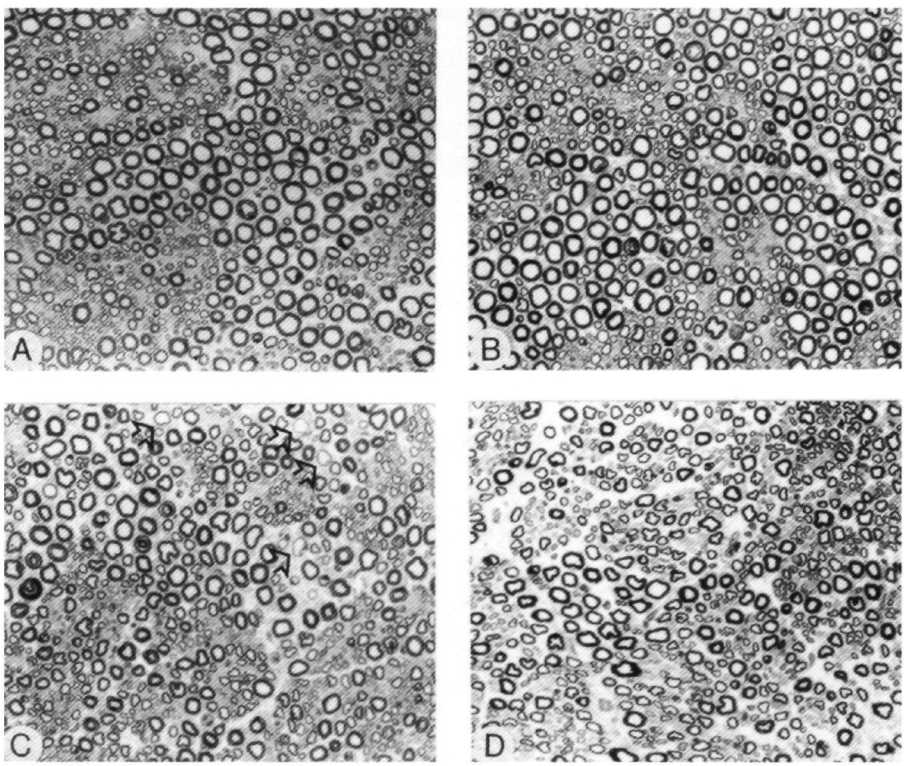

Fig. 1. A. Control nerve. B. Expanded nerve (proximal part) without morphological changes. C. Expanded nerve (middle part) showing some edema and several thinly remyelinated axons (arrows). D. Expanded nerve (distal part) showing some edema (x250).

Teased fibers

Myelin loss and remyelination was largely restricted to the paranodal region of large-diameter fibers (Fig. 2). The frequency of paranodal changes, i.e. widened nodes (C) and remyelinated nodes (F_C), ranged from 6.4 to 38.6% (mean 27.8%) (Table 1).

Table 1. Classification of teased fibers in % of total fiber number.

		Teased fibers [1]						
Procedure X/Y	Nr.	A	C	F_C	D	F_D	E	H
$0cm^3/2wk$	63	79.8	4.8	1.6	3.2	1.6	4.0	0
$0cm^3/6wk$	115	40.9	6.1	26.1	0.9	9.6	2.6	13.9
$5cm^3/2wk$	104	55.8	35.6	1.0	1.0	2.9	0	3.8
$5cm^3/6wk$	134	73.9	8.2	10.4	0	1.5	0.7	5.2
$10cm^3/2wk$	227	50.0	6.0	30.5	0	0.5	4.0	9.0
$10cm^3/6wk$	132	37.9	9.1	29.5	0.8	4.5	2.3	15.9
$15cm^3/2wk$	95	50.5	12.6	9.5	0	0	4.2	23.2
$15cm^3/6wk$	97	36.1	12.4	18.6	0	1.0	3.1	28.9
		Normal	Paranodal		Segmental		Axonal	
		53.1	27.8		3.0		15.1	

[1]X: Expander volume in cm^3; Y: Duration of full expansion in weeks; A: Normal; C: Paranodal widening; F_C: Paranodal remyelination; D: Segmental demyelination; F_D: Segmental remyelination; E: Axonal degeneration; H: Renegerated fiber (modified from Dyck, 1975).

Paranodal changes occurred at almost all nodes of Ranvier and nodal gaps of > 100 μm were not uncommmon. The widened nodes of Ranvier were remyelinated from adjacent internodes or intercalated segments, sometimes with transnodal overgrowth of myelin (Fig. 3). Complete demyelination or remyelination of the internodes was occasionally present. Linear rows of myelin ovoids indicative of axonal degeneration occurred in no more than 4.2% of teased fibers. Regenerated fibers were not observed in the first two weeks after insertion of

the expander, but occurred more frequently later on. Several regenerated fibers showed widening of nodes or paranodal remyelination. Paranodal invagination with occlusion of the nodal gap, indicative of acute nerve compression [17] was not observed. "Tadpole-like" appearances of internodes or paranodal swellings as seen in chronic nerve compression [18] occurred sporadically.

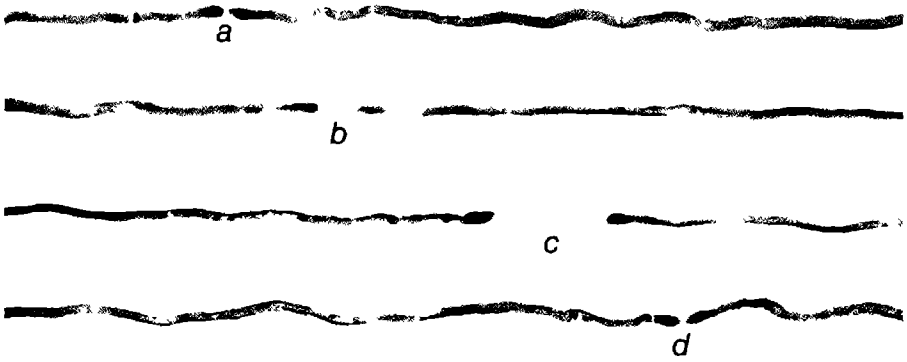

Fig. 2. Consecutive segments of teased fiber from expanded nerve. a and d: normal nodes of Ranvier. b: widened node. c: remyelination of widened node (x130).

Fig. 3. Remyelinated paranodal area with slight transnodal overgrowth of myelin (x190).

Quantitative analysis

Morphometrical analysis did not reveal a change in total fiber number or axon diameter as compared to the control side. The number of degenerating axons was not significant: less than 2.6% in 92.5% of the nerves (Table 2). Demyelination, remyelination or axonal degeneration

occurred occasionally in the middle and distal parts. Remyelination and axonal degeneration increased significantly, but demyelination did not change from the middle to the distal part (Table 3).

Table 2. Percentage of degenerating axons in distal transverse sections of expanded nerves (n=40).

Expanded nerves (% of total)	Degenerating axons (% / cross nerve section)
37.5	< 0.3
27.5	0.3 - 1.3
27.5	1.3 - 2.6
7.5	> 2.6

Table 3. Mean values of grade of pathology (G_i) ± SD for demyelination, remyelination and axonal degeneration in control nerve and examined levels of expanded nerve (n=40). Paired comparison T-test for significance of differences in parameters between examined levels.

	Morphological characteristics		
Nerve section	Demyelination	Remyelination	Axonal degeneration
Control	0	0.01 ± 0.04	0.05 ± 0.29
	n.s.	**n.s.**	**n.s.**
Proximal	0.02 ± 0.12	0.15 ± 0.45	0.07 ± 0.23
	*	****	****
Middle	0.19 ± 0.39	0.97 ± 1.10	0.62 ± 0.62
	n.s.	**	****
Distal	0.31 ± 0.52	1.62 ± 1.22	1.67 ± 1.10

* $P < 0.05$, ** $P < 0.01$, **** $P < 0.001$, **n.s.**: not significant.

Nerve elongation, rate of elongation or neurophysiological parameters did not correlate with any morphologic parameter. Edema, evident in an increase in TTFA, was more pronounced in the middle part as compared to the proximal and distal parts (Table 4). IL_m and IL_{sum} in relation to fiber diameter did not change significantly as a result of expansion (Fig. 4).

Table 4. Mean TTFA ± SD in mm^2 of control nerve and examined levels of expanded nerve (n=40). Paired comparison T-test for significance of differences in TTFA.

	TTFA		
Nerve section	Control nerve		Expanded nerve
Proximal	_		1.71 ± 0.36
			n.s.
Middle	1.42 ± 0.14	****	1.76 ± 0.48
			***
Distal	_		1.36 ± 0.39

**** $P < 0.0001$, *** $P < 0.005$, **n.s.**: not significant.

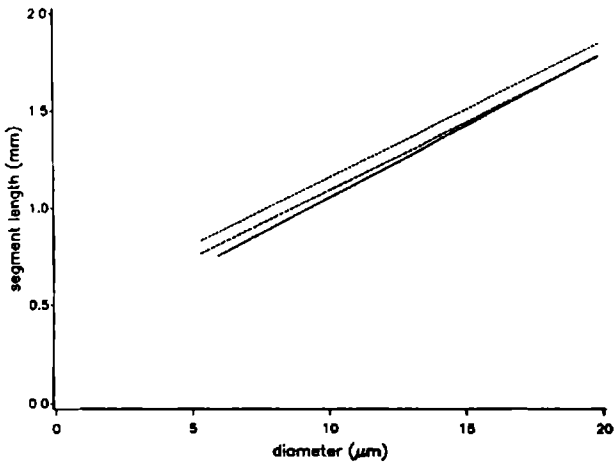

Fig. 4. Relationship between internodal (segment) length of control nerves (—), ILm (--) and ILsum (-) of expanded nerves versus fiber diameter.

Discussion

Only a few reports are available on morphological changes in rat sciatic nerves elongated by tissue expanders [5, 7, 14]. Milner and Wilkins [14] attained an elongation of 32% within 15 days, resulting in edema with vacuolation and loss of myelin. Endo and Nakayama [5] attained an elongation of 88% in eight weeks. While edema was present at all levels, vacuolation and convolution of myelin sheaths were restricted to the middle and distal segments. Hall et al. [7] obtained an increase in length of 30-40% in 14 days. Histological examination of two animals showed varying degrees of edema and axonal degeneration.

In the present study, nerve elongation was very slow: 4.2% (± 1.38) per week, whereas ischemia was avoided by LDF [27]. The morphological changes can be summarized as three phenomena: (1) paranodal widening and remyelination; (2) axonal degeneration and regeneration, associated with some segmental demyelination and remyelination; (3) edema. Whereas edema was a generalized phenomenon, the other morphological changes were restricted to the middle and distal parts of the expanded nerves.

Paranodal widening was an early and constant feature of expanded nerves, soon followed by remyelination of the node. IL_m and IL_{sum} in relation to fiber diameter tended to increase. However, the changes were not statistically significant probably due to large dispersion. Comparable changes at the nodes of Ranvier were observed by Dyck et al. [4] at the cuff edges after compression. They suggested three processes involved in lengthening of the nodes: (1) lengthening of the axons due to displacement of axonal contents; (2) detachment of myelin at the nodes of Ranvier; (3) cleavage and longitudinal displacement of myelin. Because in our model only few changes indicative of nerve compression were found, paranodal widening probably resulted from stretch of the axon. In this view, nerve expansion causes elongation of the axon, while the apparently more rigid myelin sheath lags behind and detaches at the node of Ranvier. This is in concurrence with previous work [21] demonstrating different elastic properties of axon and myelin sheath. The widened nodes of Ranvier become subsequently remyelinated from adjacent internodes or intercalated segments. Dilatation of the Schmidt-Lanterman incisures in the expanded nerves is probably not a fixation artefact, as expanded and control nerves were identically processed. The Schmidt-Lanterman incisure is the first

structure to change in neuropathy [23] and may represent a shearing defect in response to physiological stress [22]. The Schmidt-Lanterman incisures allow for some plasticity in the myelin sheath and enable the internodes to elongate [6]. They may provide a site for incorporation of new material into the myelin sheath resulting in longitudinal myelin growth [2].

A nonsignificant amount of degenerated axons occurred in most expanded nerves. Mere placing of the expander, which forced the sciatic nerve to an elongation of 10% (± 0) in two weeks [27], already resulted in some axonal degeneration. Further elongation of the nerve by gradual inflation of the expander did not yield a significant increase in axonal degeneration. Thus, LDF controlled expansion allows rabbit sciatic nerve to gain length without axonal degeneration.

Endoneurial edema is a common phenomenon in expanded nerves [5, 7, 14]. Nerve edema may be induced by a variety of mechanisms, e.g. axonal degeneration or altered vascular permeability [19]. Chronic endoneurial edema increases intercapillary distances, and may result in endoneurial hypoxia, especially in the subperineurial areas [12]. In the present study, increase in TTFA did not correlate with axonal degeneration. Moreover, the subperineurial regions were not preferentially affected. Apparently, edema did not result in significant endoneurial hypoxia.

The absence of morphological changes typical for ischemia [8, 16] supports the concept of intact NBF during LDF controlled expansion. In addition, a generalized pressure injury by the expander was excluded by lack of morphologic evidence [17, 18].

NCV of the expanded nerves was reduced [27]. No correlation was established between neurophysiological and morphological parameters (i.e. demyelination, remyelination or axonal degeneration). Moreover, a decrease in fiber diameter as a cause of a reduction in NCV [25], was not observed. Thus, the decrease in NCV may be related to nodal widening or changes in nodal membrane properties after remyelination [24, 25].

In conclusion, axons and myelin sheaths are capable to remodel and gain length provided that nerve ischemia is avoided during expansion.

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CHAPTER 8

PERIPHERAL NERVE ELONGATION BY LASER DOPPLER FLOWMETRY MONITORED EXPANSION: AN EXPERIMENTAL BASIS FOR FUTURE APPLICATION IN THE MANAGEMENT OF PERIPHERAL NERVE DEFECTS

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Abstract
Nerve grafting often fails to achieve optimal functional results and is always associated with donor-site morbidity. Peripheral nerve elongation by the use of a tissue expander may provide a useful adjunct in the management of segmental nerve loss. In the present study rabbit sciatic nerve (n=40) was elongated by expansion while nerve blood flow was monitored using laser Doppler flowmetry (LDF). Elongation was possible up to 40% with preservation of clinical nerve function. Nerve conduction velocity (NCV) of the expanded nerves decreased in a linear relation to elongation. The reduction in NCV may be secondary to the observed widening of the nodes of Ranvier and altered membrane properties after remyelination. Demyelination and remyelination of whole internodes, and axonal degeneration occurred only sporadically. Thus, LDF monitored expansion provides a safe method for elongation of intact rabbit sciatic nerve while nerve function and axonal continuity are preserved. Further studies are needed before clinical use is considered. This technique may represent a favourable alternative to nerve grafting for the treatment of peripheral nerve defects.

Introduction

Military conflicts, civilian violence, and industrialization of society have increased the number of patients requiring peripheral nerve reconstruction. If a peripheral nerve is cleanly transected, immediate primary suture represents the best means of repair. In more extensive injuries or delayed nerve repairs, often a nerve gap has to be overcome. Both experimental and clinical research have shown that tension at the suture line inhibits nerve regeneration and will jeopardize the ultimate functional result[1,2]. When primary nerve repair cannot be performed without tension at the suture line, interposition of a nerve graft is needed[3]. The functional results of nerve grafting are far from excellent despite the introduction of interfascicular nerve grafting[4] and further refinements in microsurgical techniques. In addition, harvesting a nerve graft is associated with donor-site morbidity of scarring, sensory loss and occasionally, a painful neuroma[5]. Peripheral nerve elongation by the use of a tissue expander may provide a useful adjunct in the management of segmental nerve loss[6]. Tissue expansion was initially reported by Neumann[7]. Since then silicone tissue expanders have been used with increasing frequency for reconstruction of skin and soft tissue defects[8,9]. Recently, these materials have been applied for expansion of blood vessels[10], viscera containing smooth muscle[6] and peripheral nerves[6,11,12]. If a nerve could be elongated by this method, then the additional length could be used to overcome a nerve defect, so that primary repair with a single coaptation can be done later[12]. However, it is important to control nerve function during expansion. Nerve function and structure depend intimately on the integrity of its blood supply[13,14]. Laser Doppler flowmetry (LDF) can be used to measure nerve blood flow (NBF) in both normal and injured nerves[15,16]. LDF is also a valid method of monitoring NBF during expansion and allows avoidance of nerve ischemia[17]. The present series of experiments were designed to investigate whether peripheral nerve elongation by expansion is possible with preservation of nerve function and structure when NBF is monitored by LDF.

Materials and methods

Experimental design

Fifty adult New Zealand white rabbits, unselected for sex, weighing between 2.5 and 3.5 kg were used in this study. The rabbits were housed in individual cages and received small animal diet and water *ad libitum*. The animals were divided at random in a sham group (n=11) and an experimental group (n=39). The rabbits in the experimental group were randomly assigned to three subgroups, in relation to the instilled expander volume: 5 cc (n=12), 10 cc (n=13) or 15 cc (n=14). Ten animals were withdrawn from the study following anesthetic death (n=2), pneumonia (n=3), expander failure (n=4) or wound infection (n=1). Thus, 40 rabbits were available in four groups: sham (n=10), 5 cc (n=10), 10 cc (n=11) and 15 cc (n=9).

Surgical procedures

The animals were anesthetized using 0.5 ml/kg Hypnorm® i.m. (10 mg/ml fluanison and 0.3125 mg/ml fentanylcitrate) followed by N_2O/O_2 and ethrane inhalation. The rabbits were placed on their left side on a thermostated heating pad that maintained rectal temperature at 36-37,5°C. The right sciatic nerve was exposed using aseptic technique. Four 9/0 Ethilon® sutures were placed in the epineurium at distances of 10 mm. The sciatic nerve was gently placed in a specially designed Teflon nerve holder. The nerve holder fits snugly in a 20 cc custom-made spherical expander (CUI Corporation Carpinteria, CA, USA). The tissue expander and the procedure for LDF registration have been described before by van der Wey et al.[17] (Fig. 1). In order to facilitate a continuous recording of NBF at a fixed point on the nerve's surface, the fiber optic probe runs in a special groove in the nerve-holder at the center of the spherical nerve expander. The LDF output was measured in the experimental group using an angled tip microfibre (PF 319, Perimed, Järfälla, Sweden) connected to a Master Probe (PF 318, Perimed). The Master Probe was inserted in a laser Doppler flowmeter (Periflux 2B, Perimed). After a base-line recording of pulsatile LDF output was obtained, the microfibre was disconnected. A subcutaneous tunnel on the animal's back accommodated the fiber optics and the fill-dome. Postoperatively the rabbits were daily examined in relation to gait and toe-spread reflex (TSR). Gait was scored as normal

or impaired due to paresis or plegia of the operated leg. TSR was scored as normal, decreased or absent. The animals in the experimental group were re-operated for LDF monitored expansion of the sciatic nerve. A base-line recording of LDF output was obtained after exposure of fiber optics and fill-dome. The expander was gradually inflated with 2 to 4 cc of warm saline (approximately 30°C) while NBF was monitored by LDF. As soon as the LDF signal decreased, inflation of the expander was stopped, thus preserving intact NBF during expansion[17]. This procedure was repeated at weekly intervals until the desired expander volume of 5, 10 or 15 cc was obtained. All animals were re-operated two or six weeks following either implantation of the expander (sham group) or completion of the desired expander volume of 5, 10 or 15 cc (experimental group). Measurement of nerve elongation and neurophysiological testing was performed after removal of the deflated expander.

Measurement of elongation

Nerve expansion is defined as the increase in length of the nerve by use of a tissue expander. Nerve elongation was measured by adding up the distances between the epineurial marker sutures. The degree of elongation is the increase in length of the nerve following removal of the expander as compared with the original length expressed as a percentage.

Neurophysiological methods

The temperature of the nerves was controlled by bathing the relevant section in a saline solution of approximately 30°C. A stimulating electrode was applied proximal to the expanded region. A rectangular monophasic current pulse (duration 0.2 ms) was passed through this electrode. The stimulating voltage was set at three times the threshold voltage. A recording electrode was applied 18 mm distally for registration of the compound nerve action potential (CNAP). CNAP was quantified by its main peak latency and peak-to-peak amplitude. Combined with the interelectrode distance the nerve conduction velocity (NCV) was assessed. The same procedure was performed on the contralateral nerve as a control. The animals were sacrificed for morphological investigation after measuring nerve elongation and performing neurophysiological studies.

Morphological methods

The expanded sciatic nerve was removed immediately. Three parts of the expanded nerve were processed for microscopy studies: the first part proximal to the expander, the second part overlying the expander, and the third part distal to the expander. The contralateral nerve was used as a control. Small nerve fragments of each part were fixed in 2% glutaraldehyde buffered with sodium cacodylate pH 7.4, postfixed in 2% osmiumtetroxide in Palade buffer pH 7.4, and embedded in Epon 812 after dehydration in alcohol. Semithin 1-μm-thick transverse sections were stained with 1% toluidine blue. One part of the sciatic nerve just distal to the expanded region was fixed in 2% calciumchloride and 4% formaldehyde and postfixed in 1% osmiumtetroxide in 0.1 M phosphate buffer pH 7.4. Single myelinated fibers were teased under the dissecting microscope and classified according to Dyck[18]. The total number of degenerating fibers was counted in the distal part of the expanded nerves. Fiber densities and diameter distributions of myelinated axons were determined from electron microscopic photographs (1500 ×) of ultrathin sections of the expanded and control nerve of six animals using a Zeiss TGZ 3 particle size analyzer.

Statistical analysis

A test for normal distribution indicated that the results were normally distributed. Data in each group are presented as mean values ± SEM. Linear regression calculations and analysis of variance were performed on the relation of nerve elongation versus expander volume, and on the neurophysiological data of the expanded nerve and its control. Paired Student's T-statistics were performed on density and total number of myelinated fibers of the expanded nerve and its control. In all statistical analyses $P<0.05$ was considered as significant.

Results

General

All forty rabbits remained healthy throughout the study without evidence of auto-mutilation, foot ulceration or contracture formation.

Nerve elongation

The nerves were found to have significantly increased in length following removal of the expanders (Fig. 2).

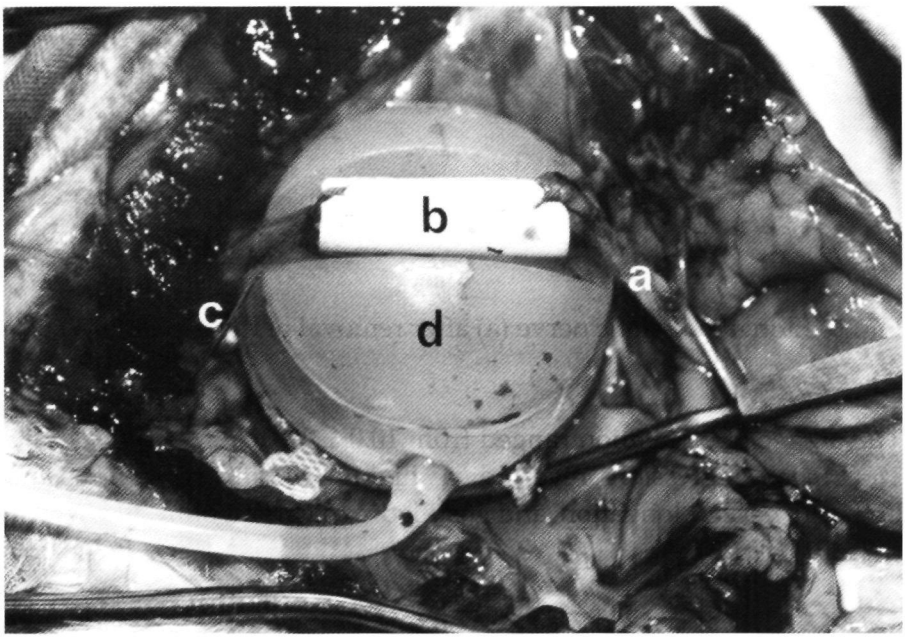

Fig. 1. Intraoperative view of the sciatic nerve (a), contained within the Teflon nerve holder (b) with fiber optics (c) in situ, overlying a fully inflated nerve expander (d).

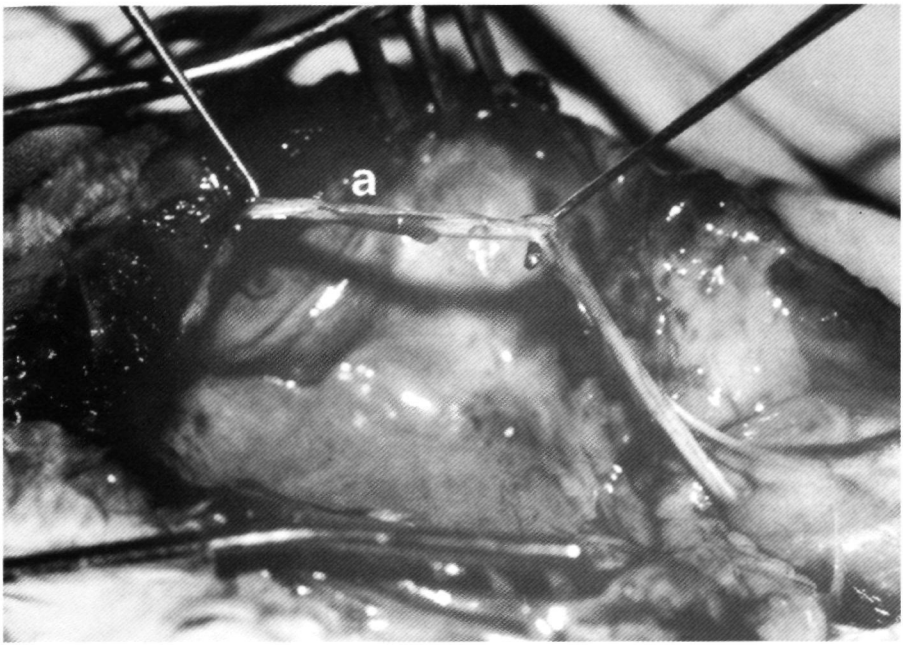

Fig. 2. Elongated sciatic nerve (a) after removal of the expander.

The degree of elongation ranged from 10.0 ± 0% to 38.6 ± 5.6% (Table 1). Linear regression revealed a highly significant relationship between nerve elongation and instilled expander volume (1.5%/cc, $p<0.001$) (Fig. 3).

Table 1: Degree of elongation (%) in relation to instilled expander volume.

	2 weeks	6 weeks
sham	10.0 ± 0	20.0 ± 2.7
5 cc	22.0 ± 1.8	21.7 ± 2.2
10 cc	26.7 ± 1.9	36.3 ± 4.3
15 cc	34.2 ± 2.7	38.6 ± 5.6

Results are expressed as mean values ± SEM

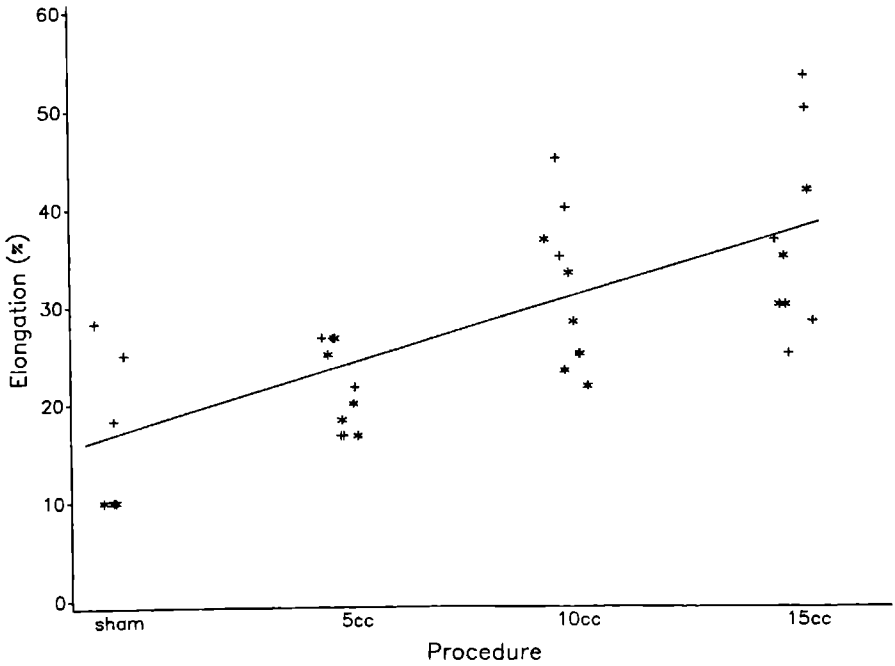

Fig. 3. Degree of elongation (%) in relation to instilled expander volume (cc). Elongation = 16.0 + (1.4 x expander volume) ($r=0.5$, $p<0.001$). Symbols: *: Restperiod of two weeks; +: Restperiod of six weeks.

Nerve function

Any disturbance in gait or TSR occurred initially after insertion of the expander and did not present in the course of the expansion procedure. Gait remained normal in 29 rabbits (72.5%). Eleven animals (27.5%) had a paresis of the operated leg for an average of 19.4 ± 3.2 days. TSR was decreased in 23 (57.5%) and absent in 17 rabbits (42.5%). TSR recovered completely in all animals after an average period of 22 ± 3.3 days. Time to full recovery of gait or TSR was not significantly related to the degree of elongation (data not shown).

Neurophysiology

The data are presented in Fig. 4 and Tables 2 and 3. Insertion of the expander resulted in a highly significant decrease of NCV ($p<0.001$) and increase of threshold ($p<0.001$), and a significant decrease of

amplitude ($p<0.005$). NCV decreased significantly in relation to elongation (0.8% per percent elongation, $p<0.01$). Amplitude and threshold did not change in relation to elongation. NCV, amplitude and threshold did not change from two to six weeks after completion of the desired expander volume (Table 2).

Table 2: Compound nerve action potential.

NCV (m/sec)

	weeks	expander	contralateral control
sham	2	30.7±3.2	44.4±6.0
	6	20.8±10.7	37.1±2.1
5 cc	2	31.4±3.3	41.8±2.8
	6	37.5±3.6	48.1±1.3
10 cc	2	28.3±2.0	42.0±1.6
	6	26.2±2.0	46.5±1.3
15 cc	2	21.1±1.6	44.6±4.0
	6	34.7±13.3	69.9±3.0

Threshold (mA)

	weeks	expander	contralateral control
sham	2	0.7±0.3	0.2±0.1
	6	0.4±0.0	0.1±0.0
5 cc	2	0.9±0.3	0.3±0.1
	6	0.6±0.1	0.4±0.1
10 cc	2	1.2±0.2	0.3±0.1
	6	1.4±0.4	0.9±0.3
15 cc	2	1.7±0.4	0.4±0.2
	6	1.6±0.6	0.2±0.0

Amplitude (mV)

	weeks	expander	contralateral control
sham	2	0.3±0.2	0.9±0.4
	6	1.5±0.6	0.3±0.1
5 cc	2	2.6±0.4	0.9±0.2
	6	1.3±0.4	1.2±0.3
10 cc	2	2.1±0.3	0.7±0.2
	6	1.0±0.3	0.5±0.2
15 cc	2	1.9±1.0	0.6±0.2
	6	0.5±0.2	1.8±0.4

Results: mean ± SEM

Table 3: NCV, amplitude and threshold in relation to instilled expander volume and degree of elongation.

Compound nerve action potential

	endvolume		elongation
	sham value (%)	slope (%/cc)	slope (%/%)
NCV	82****	-1.8****	-0.8**
Amplitude	42***	0.8ns	-0.5ns
Threshold	48****	-0.8ns	-0.7ns

Values represent the ratio of expander and control values (NCV, amplitude) or the ratio of control and expander values (threshold) times 100%. These ratios have been chosen to obtain values less than 100% for all three parameters. Symbols: *: P<0.05; **: P<0.01; ***: P<0.005; ****: P<0.001; ns: not significant.

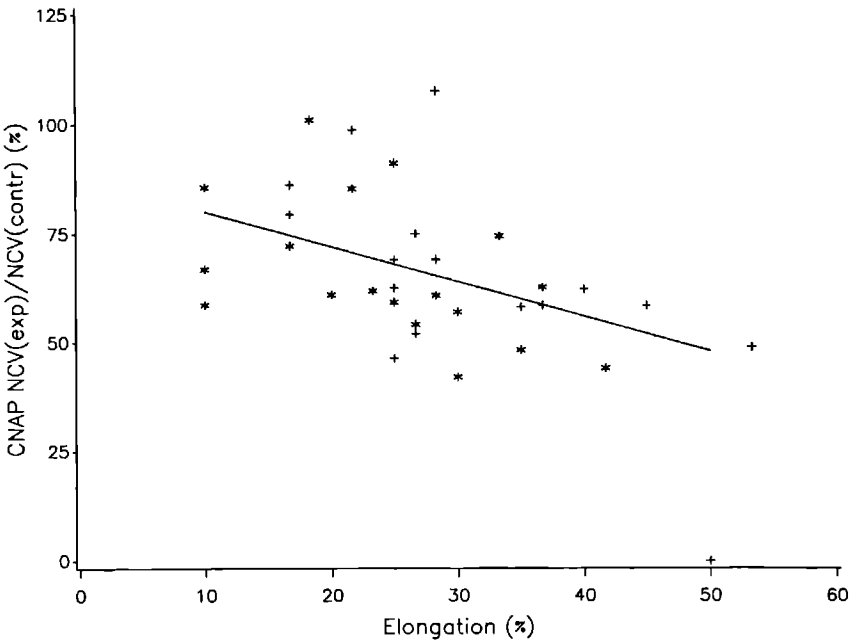

Fig. 4. NVC (CNAP), presented as the quotient of expander and control values (x100%), in relation to degree of elongation (%). NCV = 87.8 - (0.8 x elongation) (r=0.2, p<0.01). Symbols: *: Rest-period of two weeks; +: Restperiod of six weeks.

Morphology

The perineurium was of normal thickness and structure, whereas epineurial thickening occurred in the middle part. Some endoneurial edema was present at all levels (Fig. 5). No nerve fiber changes were observed proximal to the expander. Demyelinated and thinly remyelinated axons were seen occasionally in the middle and distal segments (Fig. 5).

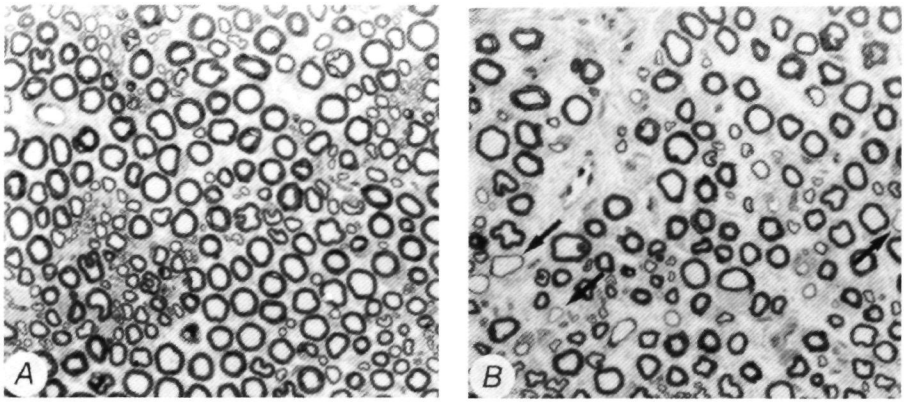

Fig. 5. (A) Control nerve.
(B) Middle part of expanded nerve showing some edema and several thinly remyelinated axons (arrows) (x250).

Axonal degeneration occurred sporadically in these parts. Teased fiber examination revealed widening of the nodes of Ranvier with subsequent remyelination of the exposed nerve segments in mainly large-diameter fibers (Fig. 6).

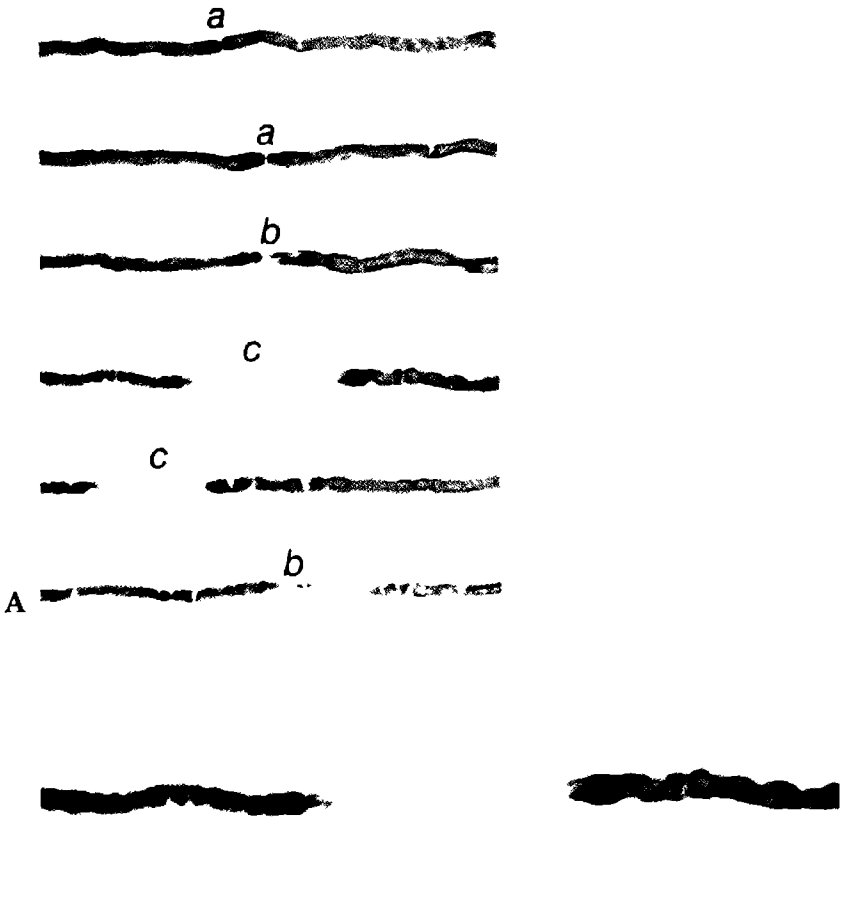

Fig. 6. (A) Consecutive segments of teased fiber from expanded nerve with normal nodes (a), widened nodes (b), and remyelinated nodes of Ranvier (c) (x130).
(B) Higher magnification of remyelinated node of Ranvier (x160).

Signs of chronic nerve compression, e.g. redundant myelin loop formation, "tadpole-like" appearance of internodes or paranodal swelling[19] occurred sporadically. Morphological changes typical for ischemia[20,21] or acute nerve compression[22], were not observed. Axon diameter distributions did not reveal a decrease in axon diameter (Fig. 7).

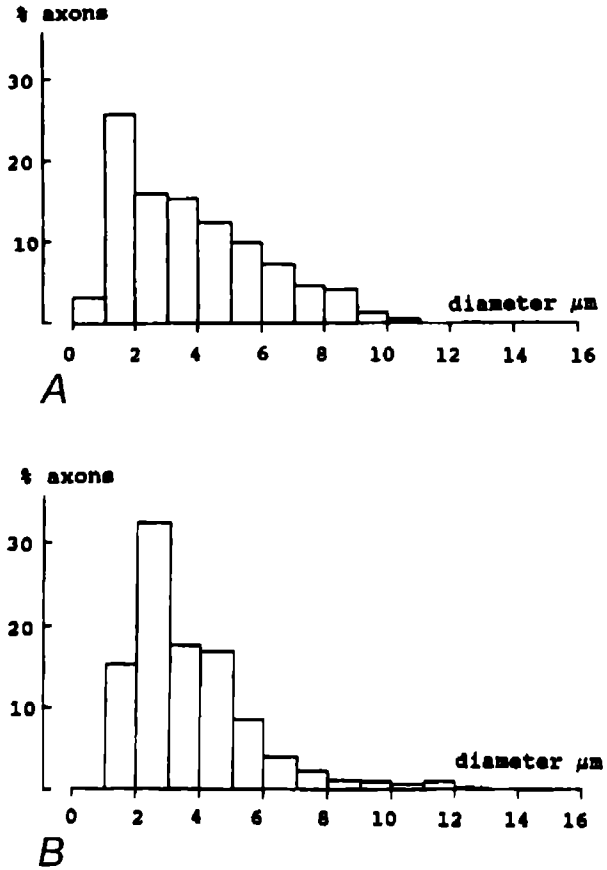

Fig. 7. Axon diameter distributions of myelinated fibers.
(A) Control nerve.
(B) Middle part of expanded nerve.

Density and total number of myelinated fibers did not change significantly in the expanded nerves as compared to the control nerves (Table 4). The number of degenerating axons was less than 2.6% in 92.5% of the nerves (Table 5).

Table 4: Density and total number of myelinated fibers (MF) in expanded nerves and control nerves (n=6).

	Density (MF/mm^2)	Total number of MF
Expanded nerves	11603 ± 1215	17865 ± 1708
Control nerves	11199 ± 1559	15126 ± 2907

Results are expressed as mean values ± SEM

Table 5: Percentage of degenerating axons in distal transverse sections of expanded nerves (n=40).

Expanded nerves (% of total)	Degenerating axons (% / cross nerve section)
37.5	< 0.3
27.5	0.3 - 1.3
27.5	1.3 - 2.6
7.5	> 2.6

Discussion

The present study revealed that rabbit sciatic nerve can be elongated up to 40% by means of a tissue expander. Mobilization of the nerve and mere placing of the expander in the sham operated animals already resulted in an increase in length of the nerve of 10% after two weeks and 20% after six weeks. The degree of nerve elongation by inflation of the expander was linearly related to the instilled expander volume.

Nerve function in relation to gait and TSR remained intact, or recovered completely within three weeks. Nerve stretching adversely affects NBF[23,24]. In our model, mobilization of the nerve and insertion of the expander resulted in an increase in length of the nerve of 10% in two weeks, safely below the critical ischemia-limit of 15% elongation[23,24]. Extrinsic circulation remained intact during expansion, in view of preservation of the small fluctuations in the LDF signal that are indicative of arteriolar contractions in the extrinsic system[17]. Moreover, morphological changes typical for ischemia[20,21] were not seen. Thus, LDF monitored expansion preserves NBF but may still have a certain pressure effect on the nerve. Local pressure may result in an acute deterio-

ration of nerve function. The effect of compression on a nerve has been related to secondary ischemia by the occlusion of small vessels[25], and to a pressure gradient in the nerve between its compressed and uncompressed parts resulting in displacement of the nodes of Ranvier[22,26]. Histological examination of the expanded nerves revealed only few changes attributable to nerve compression[19]. In contrast, endoneurial edema and nodal widening with subsequent remyelination were early and constant features. Thus, the temporary deterioration in clinical nerve function is probably a result of a conduction block during the early stages of paranodal demyelination[27].

NCV of the expanded nerves decreased in a linear relation to elongation. CNAP amplitude was reduced, and the threshold for CNAP stimulation was increased after insertion of the expander. The increased threshold is probably related to residual capsule and epineurial thickening induced by the expander[28]. A reduced amplitude may result from a decrease in number or diameter of active nerve fibers[29,30], but these changes could not be demonstrated in our study. Consequently, the decrease in amplitude may be secondary to the observed relatively mild general NCV decrease without any nerve fiber blocking[31]. NCV reflects a number of parameters and is not determined by any single structural characteristic. Decreases in fiber diameter or myelin thickness have significant effects on NCV[32,33]. NCV is also dependant on internode distance, with a broad maximum centered around the value observed in normal peripheral nerve fibers[32]. The influence of paranodal demyelination on impulse conduction appears strongly dependent on the properties of the exposed paranodal axolemma. Disruption of the axon-myelin sheet attachment, which normally seals off the internodal from the nodal axolemma, exposes inexcitable membrane and slows depolarization and impulse conduction[32,33]. In our study, a decrease in fiber diameter could not be demonstrated, whereas demyelination and remyelination of whole internodes occurred only sporadically. In contrast, nodal changes i.e. widening of the nodes of Ranvier with subsequent remyelination of the exposed nerve segments were the most striking and constant features. In conclusion, the observed reduction in NCV may result from nodal widening and changes in nodal membrane properties after remyelination[32,33,34].

A peripheral nerve can be elongated preoperatively by the use of a tissue expander. Manders et al.[6] reported the clinical use of a tissue

expander in the repair of a median nerve gap of 3 cm with encouraging early results. The nerve expansion process was well tolerated without any discomfort to the patient[6]. The rationale for nerve expansion and delayed primary repair versus conventional nerve grafting in the management of peripheral nerve defects is evident. In case of nerve expansion and primary repair, the regenerating axons have to traverse only a single coaptation to reach the end organ, whereas in nerve grafting, they have to cross two repair sites, leading to a considerable loss and disarray of fibers distal to the graft[6]. Moreover, nerve expansion and repair obviates donor-site morbidity associated with harvesting a graft from a functioning nerve somewhere else[6,12]. Proximal nerve expansion and delayed primary suture of segmental canine sciatic nerve defects yielded neurophysiological and functional results comparable to conventional nerve grafting[12]. However, nerve function was not controlled during expansion. Monitoring NBF during expansion is important because nerve function depends intimately on the integrity of its vascularization[13,14]. If elongation of the nerve is performed too quickly, nerve ischemia results in axonal degeneration. Moreover, ischemia induces endoneurial fibrosis and will inhibit the outgrowth of regenerating axons[35]. LDF is a valid method of monitoring NBF during expansion[17]. Using this technique, significant elongation of rabbit sciatic nerve up to 40% is possible with preservation of clinical nerve function. The associated decrease in NCV, secondary to paranodal changes, does not have any clinical relevance and reverses with time[27,28]. More importantly, nerve lengthening by LDF monitored expansion is feasible with preservation of axonal continuity.

In conclusion, LDF monitored expansion provides a safe method for elongation of *intact* rabbit sciatic nerve while nerve function and axonal continuity are preserved. Further studies are needed to investigate whether this technique can also be applied to elongate *injured* nerves. In addition, the functional results of LDF monitored expansion and delayed primary repair need to be evaluated for the reconstruction of segmental nerve defects. If they are comparable or even superior to those of conventional nerve grafting, the way to clinical application seems to be opened. Caution must be used in extrapolating these results to humans. Moreover, discomfort to the patient during nerve expansion needs to be further evaluated. However, this method may provide a useful adjunct in the management of peripheral nerve injuries.

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CHAPTER 9

CONCLUSIONS

The aim of the present study was to investigate whether nerve elongation by expansion was possible with preservation of nerve function and structure when nerve ischemia was avoided by laser Doppler flowmetry (LDF). Using this method, rabbit sciatic nerve was elongated up to 40% with only temporary loss of clinical nerve function. Nerve conduction velocity of the expanded nerves decreased in a linear relation to elongation as a result of widening of the nodes of Ranvier and changes in nodal membrane properties after remyelination of the exposed nerve segments. In conclusion, LDF controlled expansion provides a safe method for elongation of intact rabbit sciatic nerve while nerve function and axonal continuity are preserved. Consequently, nerve expansion and repair may provide a favourable alternative to nerve grafting for the treatment of peripheral nerve defects.
Further studies are needed to investigate whether this technique can also be applied to elongate injured nerves. In addition, the functional results of LDF controlled expansion and delayed primary repair need to be evaluated for the reconstruction of segmental nerve defects. If they are comparable or even superior to those of conventional nerve grafting, the way to clinical application seems to be opened.
Potential clinical applications of proximal nerve expansion and delayed primary suture include repairing a short nerve gap or a neuroma-in-continuity. Distal nerve expansion is another approach that may have advantages and needs to be evaluated. Expansion of nerve distal to a segmental injury involves elongating Büngner's bands and spares regenerating axons from expansion forces. If distal nerve expansion proves to be tolerated, it may also be used simultaneously with proximal nerve expansion for repair of substantial peripheral nerve defects. Since peripheral nerve elongation by LDF controlled expansion implies nerve lenghtening with preservation of its vascularization, it may also be applied to repair nerve defects across severely scarred recipient beds, the alternative being a vascularized nerve graft. In addition, if nerve expansion can not be applied because there is not enough space to accommodate the tissue expander (e.g. digital nerve gaps), it can be used to reduce donor-site morbidity by expansion of donor nerve prior to harvesting a graft. In this view, expansion of donor nerve may also be used to generate additional nervous tissue and serve as a 'nerve bank' for grafting of extensive injuries (e.g. brachial plexus injuries) when there is not enough donor nerve available.

SUMMARY

Peripheral nerve injuries with segmental loss of nervous tissue can pose a difficult problem for the reconstructive surgeon because direct nerve repair without tension at the suture line is often not possible. Although a wide variety of techniques have been employed to repair segmental nerve injuries, the procedure of nerve grafting is most widely accepted. However, despite the introduction of interfascicular nerve grafting and further refinements in microneurosurgical techniques, the functional results of this technique are frequently not satisfactory. In addition, the availability of donor nerve grafts can be a limiting factor in major reconstructive cases (e.g. repair of brachial plexus injuries). At the other extreme, when only a short nerve gap exists, the morbidity of harvesting a graft becomes a concern for both the patient and the surgeon. Another problem associated with conventional nerve grafting is the need for a well-vascularized soft-tissue bed for revascularization of the nerve graft.

Peripheral nerve elongation by a tissue expander may offer an alternative to nerve grafting for the management of segmental nerve loss. If a nerve is elongated by this method, the additional length can be used to overcome a nerve defect and enable a delayed primary repair. Moreover, donor-site morbidity associated with nerve grafting is avoided. Nerve expansion and repair may yield better functional results since regenerating axons have to traverse only one coaptation. However, it is very important to control nerve function during expansion. Nerve function and structure depend intimately on the integrity of its vascularization.

The first aim of the present study was to investigate laser Doppler flowmetry (LDF) as a method of monitoring nerve blood flow (NBF) during expansion. Rabbit sciatic nerve was gradually expanded with a custom-made spherical tissue expander, while NBF was monitored by LDF. All nerves exhibited a pulsatile flow. Inflation of the expander constantly resulted in a decrease in LDF signal, whereas subsequent deflation of the expander resulted in an increase in LDF output. Thus, LDF proved to be a valid method of monitoring NBF during expansion.

Consequently, this technique was applied for elongation of rabbit sciatic nerve in a chronic tissue expansion model. The tissue expander was inflated at weekly intervals while NBF was controlled by LDF in order to avoid nerve ischemia. Using this technique, nerve elongation was

possible up to 40%, whereas the increase in length of the nerve was linearly related to the instilled expander volume. Nerve function in relation to gait and toe spread reflex remained intact, or recovered completely within three weeks. Nerve conduction velocity (NCV) of the expanded nerves decreased in a linear relation to elongation without blocking of nerve fiber impulses. On morphological examination, widening of the nodes of Ranvier (paranodal widening), followed by remyelination of the nodes were early and constant features. In contrast, demyelination and remyelination of whole internodes, and axonal degeneration occurred sporadically and did not correlate with elongation, rate of elongation or neurophysiological parameters. Apparently, the model of LDF controlled nerve expansion provides for remodelling of myelin sheaths and lengthening of nerve fibers without axonal damage. The temporary deterioration in clinical nerve function is probably a result of a conduction block during the early stages of demyelination. The reduction in NCV may be secondary to nodal widening and changes in nodal membrane properties after remyelination of the exposed nerve segments.

In conclusion, LDF controlled expansion provides a safe method for elongation of the rabbit sciatic nerve while nerve function and axonal continuity are preserved. Consequently, this technique may provide a favourable alternative to conventional nerve grafting for the treatment of peripheral nerve defects.

SAMENVATTING

In de reconstructieve chirurgie vormen perifere-zenuwletsels met segmentaal verlies van zenuwweefsel een moeilijk probleem omdat primair zenuwherstel zonder spanning op de zenuwnaad vaak niet mogelijk is. Hoewel meerdere technieken zijn toegepast ter overbrugging van perifere-zenuwdefecten, is de techniek van zenuwtransplantatie op dit moment de meest geaccepteerde. Deze behandeling leidt echter vaak tot onbevredigende resultaten ondanks de introductie van interfasciculaire zenuwtransplantatie en verdere verfijning van microneurochirurgische operatietechnieken. Bovendien is er vaak onvoldoende donorzenuw beschikbaar bij de behandeling van uitgebreide zenuwletsels (b.v. plexus brachialis letsels). In het andere uiterste, wanneer er sprake is van slechts een klein zenuwdefect, baart de donorplaatsmorbiditeit gepaard aan het oogsten van een zenuwtransplantaat een zorg voor zowel de patiënt als de chirurg. Een ander probleem geassocieerd met conventionele zenuwtransplantatie is de behoefte aan een goed gevascullariseerd wondbed voor revascularisatie van het transplantaat.
Perifere-zenuwverlenging door middel van een tissue expander kan een goed alternatief bieden voor zenuwtransplantatie bij de behandeling van perifere-zenuwdefecten. Wanneer een zenuw op deze wijze verlengd wordt, kan de gewonnen lengte gebruikt worden ter overbrugging van een zenuwdefect zodanig dat een uitgesteld-primaire zenuwreconstructie mogelijk is. Bovendien wordt de donorplaatsmorbiditeit geassocieerd met zenuwtransplantatie vermeden. De techniek van zenuwexpansie en herstel leidt mogelijk tot betere functionele resultaten omdat regenererende axonen slechts één zenuwnaad hoeven te passeren. Het is echter van groot belang om de zenuwfunctie tijdens expansie te controleren. De integriteit van een zenuw is nauw gerelateerd aan een intacte vascularisatie.
De eerste doelstelling van deze studie was te onderzoeken of laser Doppler flowmetrie (LDF) toegepast kan worden voor het bewaken van de zenuwmicrocirculatie tijdens expansie. De nervus ischiadicus van het konijn werd geleidelijk geëxpandeerd door middel van een 'custom-made' tissue expander, terwijl de zenuwdoorbloeding werd gemeten met behulp van LDF. Alle zenuwen vertoonden een pulsatiele flow. Vullen van de expander leidde steeds tot een verlaging van het LDF signaal, terwijl weer laten leeglopen van de expander leidde tot een verhoging van het LDF signaal. Hieruit werd geconcludeerd dat

LDF een betrouwbare methode bleek te zijn om de zenuwdoorbloeding te controleren tijdens expansie.
Derhalve werd deze techniek toegepast bij de verlenging van de nervus ischiadicus van het konijn met behulp van een tissue expander. De tissue expander werd met intervallen van een week gevuld terwijl de zenuwdoorbloeding gecontroleerd werd met behulp van LDF ter voorkoming van zenuwischaemie. Op deze wijze werd een zenuwverlenging verkregen tot 40%, terwijl de lengtetoename van de zenuw lineair gerelateerd was aan het expandervolume. Zenuwfunctie gerelateerd aan looppatroon en 'toe spread refex' bleef intact, of herstelde zich binnen drie weken. De geleidingssnelheid over de geëxpandeerde zenuwen nam lineair af met de mate van verlenging van de zenuw zonder optreden van een geleidingsblok. Bij morfologisch onderzoek werden verwijding van de knopen van Ranvier (paranodale verwijding) en daarop volgende remyelinisatie van de knopen reeds in een vroeg stadium bij alle geëxpandeerde zenuwen waargenomen. Segmentale demyelinisatie en remyelinisatie en axonale degeneratie kwamen daarentegen slechts sporadisch voor en correleerden niet met de mate van zenuwverlenging, de snelheid van verlenging of de neurofysiologische parameters. Blijkbaar leidt LDF gecontroleerde zenuwexpansie tot het remodelleren van myelinemembranen en verlenging van zenuwvezels zonder schade aan te brengen aan axonen. De tijdelijke vermindering van klinische zenuwfunctie berust waarschijnlijk op een geleidingsblok tijdens de vroege stadia van demyelinisatie. De geleidingsvertraging is waarschijnlijk een gevolg van de paranodale verwijding en veranderingen van membraanstructuur rondom de knopen van Ranvier na remyelinisatie.
Uit dit onderzoek wordt geconcludeerd dat LDF gecontroleerde expansie een veilige methode is om perifere zenuwen te verlengen met behoud van zenuwfunctie en axonale continuïteit. Derhalve kan deze techniek een goed alternatief bieden voor conventionele zenuwtransplantatie ter behandeling van perifere-zenuwdefecten.

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CURRICULUM VITAE

The author of this thesis was born in Utrecht in September 1959. His secondary school education was obtained at College Blaucapel, Utrecht. In 1978 he went to medical school at the Erasmus University, Rotterdam. From February to August 1981, he was associated as a research assistent with the Division of Artificial Organs of the University of Utah, U.S.A. (Prof. Dr. W.J. Kolff). From October 1983 to February 1984, he worked at the Division of Cardiothoracic and Vascular Surgery of the University of Utah Medical Center, U.S.A. (Prof. Dr. W.C. DeVries). He graduated from medical school on June 1985. September 1985 he started his general surgical training at the Department of General Surgery of the St. Antonius Hospital, Nieuwegein (Dr. T.J. Bast). From January 1988 to October 1989 he gained additional experience in thoracic and vascular surgery at the Department of Thoracic Surgery of the St. Antonius Hospital, Nieuwegein (Drs. F.E.E. Vermeulen) and traumatology at the Department of General Surgery of the Westeinde Hospital, The Hague (Dr. R.K.J. Koumans). October 1989 he started his definitive trainig in plastic and reconstructive surgery at the Department of Plastic and Reconstructive Surgery of the University Hospital Nijmegen (Dr. J.M.H.M. Borghouts and Dr. P.H.M. Spauwen), where this research project on peripheral nerve expansion was conducted. October 1992 he was registered as a plastic and reconstructive surgeon. From December 1992 he is employed as a staffmember of the Department of Plastic and Reconstructive Surgery of the University Hospital Rotterdam (Prof. Dr. J.C. van der Meulen and Dr. S.E.R. Hovius).

STELLINGEN

behorende bij het proefschrift

PERIPHERAL NERVE ELONGATION BY LASER DOPPLER FLOWMETRY CONTROLLED EXPANSION

AN EXPERIMENTAL STUDY IN RABBITS

L.P. van der Wey

Nijmegen, 25 april 1995

I

Laser Doppler flowmetrie is een betrouwbare methode voor het bewaken van de zenuwdoorbloeding tijdens expansie van de nervus ischiadicus van het konijn (dit proefschrift).

II

De nervus ischiadicus van het konijn kan met behulp van een tissue expander significant verlengd worden met behoud van functie en axonale continuïteit, wanneer de zenuwperfusie tijdens expansie gecontroleerd wordt met behulp van laser Doppler flowmetrie (dit proefschrift).

III

Tijdens expansie van de nervus ischiadicus van het konijn treedt vertraging van de zenuwgeleiding op lineair met de mate van verlenging van de zenuw; dit berust voornamelijk op verwijding van de knopen van Ranvier (dit proefschrift).

IV

Kleine perifere-zenuwdefecten en neuromen-in-continuïteit vormen een goede indicatie voor een uitgesteld-primaire zenuwreconstructie na gecontroleerde zenuwexpansie (dit proefschrift).

V

Bij mammareconstructies door middel van tissue expansion leidt een getextureerde expander, door zijn immobiliteit, over het algemeen tot een meer anatomische vorm van de borst, waarbij de nieuwe inframammairplooi zich passief vormt secundair aan de expansie.

VI

Replantaties en revascularisaties dienen, in verband met de benodigde infrastructuur, alleen in daartoe gespecialiseerde centra plaats te vinden.

VII

The typical cleft hand is a functional thriumph and a social disaster (Flatt 1977).

VIII

Bij ernstige letsels van de extremiteiten kan een meer distaal amputatieniveau behouden worden middels vrije, gerevasculariseerde weefseltransplantaties, waarbij het amputaat altijd beschouwd moet worden als een mogelijke donorplaats (Van der Wey et al. (1993) Microsurgery 14:605-607).

IX

Er bestaat geen verband tussen siliconen borstimplantaten en het optreden van bindweefselziekten of carcinomen (Gabriel et al. (1994) New Engl J Med 330:1697-1702).

X

Vanuit reconstructief oogpunt is het beter om met het ablatieve deel van een commando-operatie 's avonds aan te vangen.

XI

Met gegist bestek zeilen komt overeen met het chirurgische principe "cut as you go."

XII

De term "vuurwerkletsels" is ongelukkig gekozen, wanneer men bedenkt dat het in veel gevallen gaat om ernstig mutilerende verwondingen die veroorzaakt worden door zelf gefabriceerde bommen.

XIII

Door het sanctioneren van de terugspeelbal bij het voetbal kan men een doelverdediger beter selecteren mede op basis van zijn voetballende kwaliteiten.

XIV

Snelheidscontroles op de Nederlandse autowegen leiden vaak tot levensgevaarlijke verkeerssituaties.